CHARACTERISTICS OF SPARKLEBERRY X BLUEBERRY HYBRIDS

By

SYLVIA J. BROOKS

A DISSERTATION PRESENTED TO THE GRADUATE SCHOOL
OF THE UNIVERSITY OF FLORIDA IN PARTIAL FULFILLMENT
OF THE REQUIREMENTS FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY

UNIVERSITY OF FLORIDA

1996

ACKNOWLEDGMENTS

The author expresses her sincere appreciation to Dr. Paul M. Lyrene for directing her graduate studies and research problems, for reviewing the manuscript, and for always providing helpful assistance to the author during her course of studies at the University of Florida in Gainesville.

Gratitude is also expressed to Dr. Gloria A. Moore for her assistance in all the isozyme work. Thanks are also expressed to the following members of the author's graduate committee: Dr. Walter Judd, Dr. Ken Quesenberry and Dr. Wayne Sherman.

The author also thanks her parents, George and Betty Blyholder, and her daughter, Amber, for their support, love and encouragement. Without them, graduate studies would not have been possible.

TABLE OF CONTENTS

Abstract of Dissertation Presented to the Graduate School of the University of Florida in Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy

CHARACTERISTICS OF SPARKLEBERRY X BLUEBERRY HYBRIDS

By

Sylvia J. Brooks

May, 1996

Chairperson: Dr. Paul M. Lyrene
Major Department: Horticultural Science

F_1 hybrids and their open-pollinated progeny from the intersectional cross Vaccinium darrowi Camp x V. arboreum Marsh were studied. The 16 F_1 hybrids that were characterized were vigorous but very low in fertility. Second generation hybrids (MIKs), obtained by open-pollination of the F_1s, were extremely variable in vigor and fertility.

A morphology study confirmed the hybrid nature of the F_1 hybrids and the MIKs, provided evidence that tetraploid highbush (hybrids of V. corymbosum) was the source of the pollen that gave rise to the MIKs, and established that V. arboreum genes were being inherited and expressed by subsequent generations. The F_1s and MIKs were intermediate between their putative parents for many flower, leaf, and

berry characteristics that were measured. Four characters that clearly showed that V. arboreum genes were being expressed in the hybrids were anther awns, large seed size, marginal glands and bracteole shape.

Isozyme analysis results were consistent with the F_1 hybrids being the progeny of V. darrowi and V. arboreum. In populations of MIKs and MIK derivatives, planted in a nursery, female fertility and pollen stainability increased dramatically from the F_1s to the MIKs. Among MIK derivatives, increasing amounts of highbush in the pedigree was correlated with increased fertility.

Female fertility, estimated in greenhouse crosses from fruit set, berry weight, number and weight of seeds, number of plump seeds per berry, and number of seedlings obtained increased with increasing highbush in the pedigree. Highbush was indicated as the most likely pollen parent of the MIKs.

Chromosome counts showed that the F_1s were diploid. Three counted MIKs were tetraploid, and one was aneuploid.

High fruit set in several crosses led to an investigation of the possibility that self-fertility and parthenocarpy were unusually high in the hybrids. Further crossing determined that the MIKs were not more self-fertile than typical southern highbush cultivars.

Softwood cuttings of juvenile V. arboreum seedlings had excellent rooting in the greenhouse. Gibberellic acid did not improve germination in V. arboreum seedlots. Survival of seedlings in a greenhouse was excellent.

CHAPTER 1
INTRODUCTION

Sparkleberry

Sparkleberry (*Vaccinium arboreum* Marsh.) has several characteristics that would be desirable in southern highbush blueberry cultivars (cultivated taxon based largely on *V. corymbosum* which contains genes from other *Vaccinium* species). One is the sparkleberry's ability to thrive on soils that southern highbush tolerate poorly. Highbush blueberries require soils that are acid, pH 3.5-5.5, low in bicarbonates, and high in organic matter. Sparkleberries grow well on highbush blueberry soils as well as on soils that are low in organic matter and have a pH as high as 6.2. Incorporation of wider soil tolerance could extend the range of the southern highbush as a cultivated crop.

Low fruit set is sometimes a problem for southern highbush. One factor that contributes to this problem is believed to be poor pollination by bees. The corolla aperture of the blueberry is narrow, and bees that do not sonicate, such as *Apis mellifera* L., have a difficult time extracting pollen and effecting pollination. Thus, non-

sonicating bees tend not to work blueberry flowers if any others are available. Sparkleberry flowers are very open; therefore it is not necessary for bees to use sonification to effect pollination. If the wider flower opening of sparkleberry could be transferred to southern highbush it could increase pollination, which could result in a higher fruit set and an increase in fruit size.

Sparkleberry x Blueberry

Two major barriers must be overcome to introgress sparkleberry genes into southern highbush. The first of these is making an intersectional cross, Vaccinium section Cyanococcus x Vaccinium section Batodendron. Intersectional crosses in Vaccinium usually fail or produce weak seedlings. The other crossing barrier is ploidy level. Sparkleberry is diploid and cultivated southern highbush is tetraploid. Within Cyanococcus, the 4x x 2x cross can be accomplished, but normally gives far fewer seedlings than 4x x 4x crosses. For example, tetraploid Vaccinium corymbosum L. can be crossed with diploid Vaccinium darrowi Camp. V. darrowi produces enough unreduced male gametes, up to 6.2% for some clones (Cockerham and Galletta, 1976), to give a reasonable number of tetraploid progeny.

In order to incorporate sparkleberry characteristics into southern highbush a simple plan was developed using V.

darrowi as a genetic bridge. Sparkleberry was crossed with V. darrowi. This was an intersectional cross, but with the same ploidy level. The progeny from this cross would then be crossed with highbush. This would be a heteroploid cross, but it was hoped that the progeny would have inherited the tendency to produce unreduced gametes. Once a fertile tetraploid was produced, the progeny would be backcrossed to cultivar-quality highbush. Plants could then be selected for desirable sparkleberry characteristics combined with the fruit qualities needed in a commercial clone.

In 1981, the first V. darrowi x V. arboreum crosses were made (Lyrene 1991). The V. darrowi parents were 'Johnblue', FL 80-61, and an intrasectional Cyanococcus hybrid (diploid Florida native V. corymbosum x V. darrowi). These plants were dug from the Horticultural Unit at Gainesville, Florida, and placed in a dark cooler at 5C. This was done to delay the flowering of V. darrowi until V. arboreum pollen became available. The plants were moved to a bee-proof greenhouse in February and March of 1981. Branches of early flowering sparkleberry were collected from Alachua and Santa Rosa Counties in north Florida. In all cases, sparkleberry was used as the pollen parent. Resulting seeds were sown on peat under intermittent mist. The seedlings were transplanted to a high density nursery in

May, 1982. Two-and-a-half years later there were 19 plants that were vigorous and were apparently hybrids. These were transferred to a 1.5 x 4-m field spacing. In 1995, 16 of the hybrids were still alive.

The F_1 hybrids grew vigorously in the field for several years without flowering. Eventually some of the clones became highly floriferous, and several began to produce some berries. These open-pollinated berries were harvested beginning in 1987. The seeds were extracted and germinated in the usual manner. Two hundred seedlings were transplanted to a high-density nursery in May of 1988. The following year the surviving plants were evaluated. Six plants were selected as expressing sparkleberry and Cyanococcus traits, being highly vigorous, having high fruit set and large berry size. These clones were the basis for the next stage of the program.

The plants resulting from the open-pollinated berries of the F_1 hybrids were designated as MIKs. MIK stands for Mother Is Known. The pollen could have come from any of three sources, diploid, tetraploid or hexaploid blueberries. The physical appearance of the MIKs indicated that they were tetraploid. Their leaves, flowers and berries were much larger than those of the diploid F_1 mother parents. In addition, the mean fertility level of the MIKs had increased dramatically over that of the F_1s. The two most likely

candidates for pollen parent were the F_1s themselves or tetraploid southern highbush. The union of an unreduced egg and an unreduced pollen grain from the diploid hybrids could produce a tetraploid progeny. The other possibility is the union of an unreduced F_1 egg and normal reduced pollen from highbush. Since the F_1s are essentially male sterile, the latter is more likely.

Research Objectives

This research was initiated to study the F_1 hybrids, MIKs, and MIK derivatives that originated from crosses of *V. darrowi* x *V. arboreum*. Specific objectives included confirming the hybrid nature of the F_1s and MIKs, determining the unknown pollen parent of the MIKs, determining if *V. arboreum* genes were being inherited and expressed by subsequent generations, and investigating several methods of propagation for *V. arboreum*. Plants were studied in the greenhouse and field.

The hybrid nature of the F_1s and MIKs was investigated through morphological studies and isozyme analysis. Many characteristics of flowers, leaves and berries were measured for *V. arboreum*, *V. darrowi*, *V. corymbosum*, F_1, and MIKs. These studies were also used to determine if *V. arboreum* genes were being incorporated and expressed in the MIKs and MIK derivatives.

The pollen source for the open-pollinated berries that produced the MIKs was explored. Morphological studies, controlled crosses, chromosome counts, and pollen stainability were used to test the hypothesis that tetraploid highbush was the pollen source.

Progeny populations of MIKs and MIK derivatives were studied in a high density nursery. Data were gathered on average bloom date, average harvest date, number of flowers, number of berries, berry weight, and pollen stainability.

Experiments were conducted to gain information on the propagation of V. arboreum. In one experiment, seeds were germinated using gibberellic acid to test for increased germination. In another experiment, juvenile softwood cuttings were tested for their rooting ability. Survival of seedlings in a greenhouse environment was also investigated.

CHAPTER 2
REVIEW OF LITERATURE

Wide Hybridization

Wide hybridization is a term that is often used but rarely defined. Hadley and Openshaw (1980) defined a wide cross as one involving different species or genera. These crosses are difficult, but not impossible to make. This degree of difficulty may be reached at different taxonomic points in different plant families. In Vaccinium, interspecific crosses in the primary gene pool, between cultivated species, can often be made with relative ease (Galletta, 1975; Lyrene and Ballington, 1986; Moore and Ballington, 1990). Intersectional hybridization is possible between certain sections, but difficult.

There are 4 major reasons for using wide hybridization in a breeding program. They are 1) transfer of 1 or several characteristics, 2) expression of characteristics not present in either parent, either novel or beyond the range of the parents, 3) production of new alloploids, and 4) exploration of the relationship of one species to another (Briggs and Knowles, 1967).

Hybrids are produced when reproductive isolation barriers are overcome. Isolation barriers can be divided into 2 major categories, internal and external (Stebbins, 1950). The external barriers are geographic, ecological, temporal and seasonal, and mechanical. All of these can be overcome by the breeder with relative ease. The internal barriers are prevention of fertilization, hybrid inviability or weakness, hybrid sterility, failure of flowering, and inviability and weakness in the F_2. Many excellent reviews have been written about wide hybridization, isolation barriers and methods to overcome them (Briggs and Knowles, 1976; Hadley and Openshaw, 1980; Hermsen, 1992; Stebbins, 1950, 1958). The reader is referred to these for details not described here.

Wide Hybridization in Blueberries

Overcoming Barriers to Wide Hybridization

Although it is relatively easy to hybridize different species within section Cyanococcus, it is not effortless. The major problems are nonsynchronous flowering, heteroploidy, genetic divergence among the species in the section, and lack of progeny.

Species with nonsynchronous flowering can be induced to flower in unison. This is achieved by shuttling potted

plants between cold chambers and warm greenhouses. Plants can be held in a cold chamber until the chilling requirements of all the plants involved have been met. After removal to the greenhouse, most Cyanococcus species will bloom within 30 days (Lyrene and Ballington, 1986). Another method that achieves the same end is to store pollen. Blueberry pollen can be stored, refrigerated, for up to a year (Galletta, 1975).

It is important to carefully select the clones that will represent each species in a cross. Many species are reported to vary widely in individual male and female fertility. For this reason, it is well to verify the fertility level of selected clones. It has also been demonstrated that replicated crosses can give widely divergent results. This is probably due to genetic variation within species (Galletta, 1975; Lyrene and Ballington, 1986).

Reciprocal differences have been demonstrated in V. darrowi crosses and in heteroploid crosses. The success of tetraploid x diploid crosses usually relies on the production of unreduced gametes by the diploid. The production of unreduced gametes varies between individuals within a species and between species (Galletta, 1975; Lyrene and Ballington, 1986; Ortiz et al., 1992). Using the diploid as the pollen source allows the breeder to determine

the level of 2n gamete production via pollen staining. Vaccinium corymbosum x Vaccinium ashei Reade crosses also have shown reciprocal differences. The tetraploid x hexaploid cross, according to Galletta (1975), is more successful or only successful if the tetraploid is used as the female parent. Lyrene (1988) found no significant difference for direction of the cross in the number of seedlings produced per pollinated flower. There was, however, a large difference for direction of cross in percent fruit set and plump seeds per berry.

Some species are extremely difficult to cross. Gene exchange between these species can sometimes be achieved by genetic bridging. This approach involves a series of crosses among 3 to 4 species. Although the desired species are never directly crossed, their genomes (or partial genomes) are combined in 1 organism (Hadley and Openshaw, 1980). This technique is often used in heteroploid crosses. For example, diploid Vaccinium elliottii Chapman is very difficult to cross with tetraploid V. corymbosum. Vaccinium elliottii will cross with diploid V. darrowi, yielding fertile diploid hybrids. Compared to V. elliotii, these hybrids are relatively easy to cross with V. corymbosum. The most likely explanation for this is the low rate of 2n gamete production found in V. elliottii (Lyrene and Ballington, 1986).

Interspecific Hybridization

Interspecific crosses have been an integral part of blueberry breeding programs since Coville began his breeding program in 1906. His cultivars were based on V. corymbosum, but many of his early releases had Vaccinium angustifolium Aiton genes in their pedigrees (Galletta, 1975; Moore, 1966).

Homoploid interspecific crosses within section Cyanococcus are relatively easy to make and generally produce fertile, vigorous hybrids (Luby et al., 1990; Lyrene and Ballington, 1986; Moore, 1965, 1966). It was reported in the earlier literature that all homoploid crosses within this section were interfertile (Camp, 1945; Coville, 1927, 1937; Darrow and Camp 1945; Meader and Darrow, 1944). It has, however, been determined that interspecific crosses between several of the diploid species give reduced seedling numbers and/or hybrids of reduced fertility (Ballington and Galletta, 1978; Vander Kloet, 1978, 1980).

The species most widely used in blueberry breeding are V. corymbosum, V. angustifolium, V. darrowi, V. ashei, and Vaccinium constablaei Gray (Lyrene, 1993). These species have characteristics which complement and enhance each other. Vaccinium corymbosum (tetraploid) can contribute genes for shorter bloom-to-ripe periods, large berry size,

firmness, flavor, cold hardiness and variation in ripening season. Vaccinium angustifolium (tetraploid) can contribute genes for early-ripening, concentrated ripening, precocity, drought resistance, bud hardiness, productivity, sweetness, blue color, Phytophthora root rot resistance, blueberry shoestring virus tolerance, low stature and upland soil adaptation. Vaccinium darrowi (diploid) can contribute genes for low chilling, heat and drought resistance, and light blue fruit color. Vaccinium ashei (hexaploid) has genes for small fruit scar, light fruit color, excellent shelf life, bush vigor, heat and drought tolerance, tolerance to certain fungal diseases, and tolerance to upland soils. Vaccinium constablaei (hexaploid) has genes for delayed bloom, early ripening, cold hardiness, concentrated ripening, and ease of picking (Galletta, 1975; Luby et al., 1990; Lyrene, 1993; Lyrene and Ballington, 1986; Moore 1965, 1966).

Heteroploid intrasectional crosses require more effort for success than homoploid crosses, but are frequently not exceedingly difficult. Diploid V. darrowi has been crossed with tetraploid V. corymbosum to produce low-chilling cultivars. Tetraploid x diploid crosses yield mostly tetraploids due to a strong triploid block, although a few triploids are sometimes obtained. These crosses are successful due to the high number of 2n gametes produced by

V. darrowi (Luby et al., 1990; Lyrene and Ballington, 1986; Megalos and Ballington, 1987; Oritz et al., 1992).

There has been much interest in crossing tetraploid V. corymbosum with hexaploid V. ashei. Each species possesses characteristics that would improve and expand the adaptation of the other. The initial cross is easy to make, but produces pentaploids that have reduced fertility. For many years this was considered to be a dead end. More recently, studies have shown that the more fertile pentaploid hybrids can be backcrossed to V. corymbosum and V. ashei to produce progeny fertile enough for continued use in a breeding program (Laverty and Vorsa, 1991; Luby et al., 1990; Lyrene 1993; Lyrene and Ballington, 1986; Vorsa et al., 1987).

Another method of transferring valuable genes between V. corymbosum and V. ashei is to produce a synthetic triploid first. Vaccinium corymbosum is crossed with a diploid species. Triploids that produce unreduced gametes are selected from the resulting progeny. These plants are then crossed with V. ashei. The hexaploid hybrids produced by this cross are usually vigorous and fertile (Lyrene, 1993; Dweikat and Lyrene, 1989b).

Intersectional Crosses

The sectional or subgenus level is the point at which genetic differences that greatly interfere with

hybridization become obvious in Vaccinium. Most hybrids are weak, and if they survive to maturity are usually sterile. However, with some intersectional combinations, if enough parental combinations are tried, it is often possible to generate a few hybrids that are vigorous and fertile enough to be useful (Ballington, 1990; Luby et al., 1990; Lyrene and Ballington, 1986).

Coville was the first to try intersectional hybridization in Vaccinium. It is reported that he obtained viable hybrids from crossing Vaccinium stamineum Small (Vaccinium section Polycodium) with Vaccinium myrtilloides Michaux (section Cyanococcus) (Moore, 1966).

Hybrids have been successfully produced from crosses of Cyanococcus species with species in sections Polycodium (Darrow and Camp, 1945; Ballington, 1980), Vaccinium section Pyxothamnus (Darrow and Camp, 1945), Vaccinium section Vaccinium (Darrow, 1960; Pliszka et al., 1980; Rousi, 1963), Batodendron (Lyrene, 1991; Lyrene and Ballington 1986), and Vaccinium section Herpothamnus (Lyrene and Ballington, 1986). Other sections that have been crossed are Vaccinium section Myrtillus with Vaccinium section Vitis-idaea (Rousi, 1967), Vaccinium section Oxycoccus with Vitis-idaea (Ahokas, 1979) and Herpothamnus with Vaccinium section Bracteata, Vitis-idaea, and Pyxothamnus (Lyrene and Ballington, 1986).

Crosses between sections Cyanococcus and Polycodium (V. stamineum) show strong reciprocal differences. In most cases the Cyanococcus species must be used as the female parent (Ballington, 1980). Crosses of V. stamineum with V. elliottii have yielded only sterile hybrids. When V. darrowi is used as the female parent, the resulting hybrids are fertile enough to produce BC_1 and F_2 progeny (Ballington, 1980; Lyrene and Ballington, 1986).

Viable, but sterile progeny have been produced by crossing diploid species in sections Cyanococcus (V. darrowi) and Pyxothamnus (Vaccinium ovatum Pursh). A partially fertile tetraploid hybrid was obtained from V. corymbosum x V. ovatum. By crossing V. ovatum x V. darrowi, Ballington got a partially fertile amphidiploid (Luby et al., 1990).

Section Herpothamnus represented by Vaccinium crassifolium Andr. has been crossed with sections Cyanococcus, Bracteata, Vitis-idaea, and Pyxothamnus. One hybrid was produced by V. crassifolium x Vaccinium tenellum Aiton (section Cyanococcus). This partially fertile hybrid was successfully backcrossed to both parents (Luby, 1990). Crosses between sections Herpothamnus and Bracteata (Vaccinium bracteatum Thunb.) and Vitis-idaea (Vaccinium vitis-idaea L.) produced only sterile hybrids. Many seedlings resulting from the Vitis-idaea crosses were

chlorophyll deficient. Partially fertile hybrids resulted from crossing V. crassifolium (Herpothamnus) with V. ovatum (Pyxothamnus).

Hybrids were produced from crossing Vaccinium microcarpum (Turcz ex Rupr)Schmalh (section Oxycoccus) with V. vitis-idaea (section Vitis-idaea). Germination of the seeds was good in test tubes on Nitsch nutrient agar. Twenty-six of the 49 seedlings died at the cotyledon stage from red chlorosis. Growth of the seedlings that survived the winter was good, but the seedlings deteriorated the next year. The hybrid flower morphology and general plant habit were intermediate between the parents (Ahokas, 1979).

Rousi (1967) studied naturally occurring hybrids of Vaccinium myrtillus L.(section Myrtillus) x V. vitis-idaea (section Vitis-idaea). He obtained the hybrid plants from the Royal Botanic Gardens, Edinburgh. This hybrid has also been referred to as Vaccinium intermedium Ruthe. Chromosome pairing during meiosis in the pollen mother cells of 1 hybrid was very variable. The stainability of the pollen grains was 45.3%. An earlier report had estimated pollen germination for these hybrids at only 4.4%. Rousi's hybrid was vigorous and flowered abundantly. Very little fruit was produced and then only at the end of the growing season. Only 4 berries had seeds. Ten fully developed seeds were

extracted from the berries. These seeds produced normal-looking F_2 plants.

The most successful intersectional cross has been that of Vaccinium x Cyanococcus. In 1961, a hybridization program was begun in Finland using Vaccinium uliginosum L. and V. corymbosum (Rousi, 1963). The objective of the program was to incorporate increased winter hardiness and blueberry canker (Fusicoccum putrefaciens Sher.) resistance into cultivated highbush (Hiirsalmi and Lehmushovi, 1982).

Reciprocal crosses were made between wild V. uliginosum and 2 V. corymbosum cultivars, 'Rancocas' and 'Pemberton'. A total of 323 flowers were pollinated on the highbush cultivars and 172 flowers were pollinated on V. uliginosum. All the crosses produced berries with seeds. The size of the berries varied widely. However, only the seeds from berries on V. uliginosum germinated (Rousi, 1963).

The F_1 hybrids were vigorous and luxuriant (Rousi, 1963). As they aged, the hybrids began to exhibit hybrid breakdown as indicated by a decline in vigor and cold hardiness, production of few seeds and poor seed germination (Hiirsalmi, 1977; Hiirsalmi and Lehmushovi, 1982). The morphology of the hybrids was variable. For many characters they were intermediate between the parents. Some characters were more similar to 1 parent or the other, and some characters exceeded the parental ranges. Flowering on the

hybrids was low, but most flowers developed into berries. Many of the berries contained few or no fully developed seeds. Germination of the seeds was poor (Hiirsalmi, 1977). Chromosome pairing during meiosis in the pollen mother cells was examined and found to be good. Pollen stainability averaged 85.7% with a range of 74.3% - 94.8% (Rousi, 1967).

The good fertility and nearly perfect chromosome pairing in meiosis had 2 possible explanations. The first was that the chromosome sets of Vaccinium and Cyanococcus, as represented by the species used, were to a large extent homologous. The other possibility was that autosyndesis was occurring. In other words, the 2 sets of chromosomes from V. uliginosum paired only with each other, and the same occurred with the V. corymbosum chromosomes. The first possibility was the more desirable from a breeding point of view as this would allow for genetic recombination between the 2 species. If the V. uliginosum genotype was designated as UUUU and the Cyanococcus genotype as CCCC, the F_1 hybrid would be UUCC. If the U and C genomes were homologous, recombination would occur between them and the F_2 and backcross populations would show a large amount of variation. On the other hand, if the chromosome pairing was autosyndetic, the F_1 would only be able to produce UC gametes. With no recombination between the U and C genomes,

the backcross and F_2 progeny would be fairly uniform (Rousi, 1967).

In 1965, Rousi backcrossed the F_1 hybrids to highbush cultivars. When the F_1s were used as the female parent, 10 berries were produced that averaged 4.6 fully developed seeds per berry. When highbush was used as the female parent, 69 berries were produced averaging 2.9 fully developed seeds per berry. Germination of seeds from both backcrosses was good (Rousi, 1967). It appeared that the unfavorable gene combinations of the F_1 hybrids had been partially eliminated. Variation in the backcross progeny was often greater than in the F_1 hybrid population. Flowering and yield ranged from low to high, with berry weight being intermediate (Hiirsalmi, 1977). On average, characteristics were closer to V. corymbosum than to V. uliginosum (Hiirsalmi and Lehmushovi, 1982).

In field trials, 1 of the backcross progeny was clearly superior to the others. This plant resulted from the cross 'Rancocas' x (V. uliginosum x 'Rancocas'). Its vegetative characters were very similar to highbush. The yield of this clone was good, and berry quality was satisfactory. In addition, it had better winter hardiness and blueberry canker resistance than highbush. These were the very qualities that the Finnish breeding program was trying to

transfer. This clone became cultivar 'Aron' in 1982 (Hiirsalmi and Lehmushovi, 1982).

The high variability and good fertility of the backcross populations would seem to indicate that the genomes of V. uliginosum and Cyanococcus are homologous. The hybrid breakdown of many of the F_1 hybrids could be explained as genic sterility (Stebbins, 1958).

A breeding program was begun in Florida to cross section Batodendron with section Cyanococcus (Lyrene, 1991, 1993). The purpose was to introgress several desirable traits from V. arboreum into cultivated highbush. Vaccinium arboreum will grow in soils with a higher pH and lower organic matter content than highbush can tolerate. The plants are much more drought tolerant than the highbush blueberry. This species also has a much wider corolla opening than highbush. Opening up the highbush corolla could potentially improve bee pollination and thus increase yield.

In 1981 and 1982, diploid V. arboreum, representing section Batodendron, was crossed with 2 diploid species of section Cyanococcus, V. darrowi and V. elliotii. The cultivar 'Johnblue' was 1 of 3 V. darrowi genotypes used. The seedlings were germinated and planted in a high-density nursery. After 2 ½ years, plants were selected that were vigorous and apparently hybrids. As of 1996, there are 16

surviving vigorous hybrids from the V. darrowi cross and 11 from the V. elliotii cross. The V. elliottii hybrids are vigorous but produce no fruit in the field.

The F_1 hybrids from the V. darrowi x V. arboreum crosses were intermediate in morphology in many traits; leaf size and shape, degree of deciduousness, bark, inflorescences, and flower morphology. Flowering was extremely variable between the 16 plants, ranging from very little to abundant. The amount of flowering per bush and fruit set seemed to increase with age. Open-pollinated berries were harvested from the plants beginning in 1988. In 1990, 8 bushes produced berries; harvests were 2610, 585, 480, 360, 185, 20, 10, and 5 berries. These berries were small, ranging in weight from 0.15 g to 0.29 g. Small berry size is probably due in part to a low number of seeds per berry (Lyrene, 1993).

Preliminary observations of microgametogenesis have indicated that male sterility may be due to pre-meiotic problems (Lyrene, 1993).

The seed resulting from open-pollination of the F_1 hybrids were germinated, and the seedlings were planted in the field. The resulting progeny, (MIKs), were highly variable. Berry set varied from none to high, with fruit weight ranging from 0.3 g to 1.6 g. The flowers and leaves on these plants were much larger than those of their F_1

parents. This, along with their much greater fertility when open-pollinated, led Lyrene to believe that this second generation was tetraploid, rather than diploid like their F_1 parent.

Six of these so called MIKs were chosen for further evaluation. Selection was based on high vigor, above-average fruit set, above-average berry size and a clear expression of both V. arboreum and Cyanococcus characteristics. These clones were moved to a greenhouse for controlled crossing. They were pollinated with pollen from tetraploid highbush, intercrossed among each other, and selfed. Fruit set, berry size and ripening date were similar for each type of cross on a particular clone. Seed yield was lower for self-pollination than for the other 2 types of crosses. Most of the seedlings from these crosses were vigorous, although some crosses did produce many chlorotic seedlings.

Hybrid Identification

The identification of hybrids from a wide cross is often based on morphology. Unfortunately, for this purpose, hybrids are not always intermediate in morphology between their parents. After surveying the literature on hybrid morphology, Rieseberg (1995) reported that hybrids are just as likely to exhibit parental characteristics as

intermediate ones. In fact, his survey showed that hybrids in general were a mosaic of parental, intermediate and extreme (novel) characteristics. The expression of the trait in the hybrid depended on the nature of the genetic control of that particular trait and its interaction with the environment.

The frequency of extreme or novel characteristics was found to be over 10% in F_1 hybrids and over 30% in subsequent generations. Rieseberg (1995) listed 5 possible explanations for this phenomenon. They were: 1) increased mutation rate in hybrids, 2) complementary action of new combinations of alleles, 3) new regulation patterns of genes, 4) fixation of recessive alleles that were in the heterozygous form in the parents and 5) reduced developmental stability.

An example of the mosaic nature of hybrids is found in the intersection cross between Vaccinium and Cyanococcus previous discussed. Hiirsalmi (1977) reported that many of the characters measured in his hybrids were intermediate between the parents. However, some individual plants resembled 1 parent or the other in 1 to a few traits. In addition, some clones exceeded or fell short of both parents for certain characteristics.

Confirmation of the hybridity of a clone can be accomplished in several ways (Wilson, 1992). Wilson

examined hybrid indices, principal components analysis, pictorialized scatter diagrams and the character count procedure. He found scatter diagrams and the character count procedure to be the best. Scatter diagrams give a graphical presentation of the character states of individuals in the population. This presentation of the data permits an intuitive interpretation; it is easy to see intermediacy if it exists.

Hybrids can also be identified using biotechnical techniques. A primary use of isozymes is the confirmation of hybrids (Moore and Collins, 1983; Nielsen, 1985; Weeden, 1989). Hybrids are identified by the banding pattern they produce during electrophoresis. These biochemical markers, or bands, are co-dominant, so the hybrid will express a combination of the parental banding patterns. New bands are possible as a result of interactions between parental alleles.

Isozymes offer many advantages in hybrid identification (Moore and Collins, 1983; Nielsen, 1985). The process is nondestructive and virtually any tissue in the plant can be used. Hybrids can be screened at the seedling stage. Non-hybrids or undesirable genotypes can be removed at this early stage, reducing the waste of time and space on plants that would have been removed at a later date. If the isozyme marker has been linked with a desirable

morphological trait, seedlings or plants with the marker can be selected.

Self-incompatibility

Self-incompatibility is common among Vaccinium species. The structure of the flower reduces within-flower selfing (Ballington and Galletta, 1978; Eck and Mainland, 1971). The receptive surface of the stigma extends beyond the surrounding stamens and normally receives little pollen from the same flower. In addition, the sides of stigma are angled so as to deflect pollen falling from the anthers (Eck and Mainland, 1971).

There is good agreement in the literature that the diploid species and hexaploid V. ashei are partially to highly self-incompatible. Ballington and Galletta (1978) examined self-incompatibility in 4 diploid species (Vaccinium atrococcum Heller, Vaccinium caesariense Mackenzie, V. tenellum and V. darrowi). They found that self-pollinations were considerably less successful than intraspecific cross-pollinations; only 1/6 the amount of fruit was produced, 1/37 as many seeds, 1/46 as many germinated seeds and 1/52 as many vigorous seedlings were produced.

Vaccinium ashei is generally considered to be self-incompatible, although there is some variability. Ten

cultivars were studied and it was found that 4 were completely self-incompatible, 5 were partially incompatible, with fruit set after self-pollination ranging from 9% to 33%. One cultivar was self-compatible (Meader and Darrow, 1944). Another study (El-Agamy et al., 1981) of 5 cultivars found that the average fruit set for selfing was 18% with a range of 3% to 33%. Cross-pollination for these cultivars resulted in an average fruit set of 47%, with a range of 3% to 81%. The average number of seeds per berry for selfing was 1.5, with a range of 0 to 4.2. Essentially the same results were found in a survey of 19 native V. ashei. Fruit set averaged 15% after selfing and 58% after cross-pollination (Garvey and Lyrene, 1987).

Reports on the self-compatibility of V. angustifolium (lowbush) are similar to V. ashei. Aalders and Hall (1961) found that percent fruit set for self-pollination averaged 16.2%, with a range of 0 to 52.0%. Cross-pollination gave an average fruit set of 86.6% with a range of 80.6% to 90.3%. Wood (1968) undertook a study involving 92 clones of lowbush in 4 different native meadows. He also found variability in the levels of self-compatibility, but found the general level of fruit set higher than reported by Aalders and Hall. Only 5 of the clones set no fruit at all. He reported that in 1 field (50 clones), 50% of the clones had a fruit set greater than 50% after self-pollination.

Tetraploid V. corymbosum, the highbush blueberry, shows a large amount of variability in self-compatibility. Coville (1937) reported that experiments to self-pollinate highbush were a failure and he abandoned the technique. White and Clark (1939) found a wide range of variation among highbush cultivars from total self-incompatibility to total self-compatibility. El-Agamy et al. (1981) found that the average fruit set after selfing 5 cultivars and advanced selections was 67% with a range of 43% to 95%. An average of 3.9 seeds per berry with a range of 2.2 to 5.4 were produced by these selfings. Cross-pollinations for these same clones averaged 82%, with an average of 11.2 seeds per berry.

A study (Vander Kloet and Lyrene, 1985) of self-incompatibility in all ploidy levels of V. corymbosum has demonstrated several points that had confused the issue. The first was that most studies have been done with cultivars or selections from breeding programs. These clones have been selected for their ability to fruit well in commercial blocks planted with only 1 or a few clones. Therefore, they are not representative of the species as a whole. The second issue was method of self-pollination. Some researchers have placed plants in a greenhouse without insects or manual self-pollination, while others have applied self pollen to the stigma. The structure of the

flower reduces the amount of self-pollination that occurs in the absence of insects. A third point was the criteria used to determine success of the cross. Some studies have used percent fruit set, some have reported number of plump seeds, and others have reported number of seedlings produced per pollinated flower. Depending on the criteria used, there could be variation in the self-fertility level of the same self-pollination event.

In an effort to avoid these problems, Vander Kloet and Lyrene (1985) undertook a study of native populations of diploid, tetraploid and hexaploid V. corymbosum. The self-pollinations were less successful than cross-pollination in every experiment for each ploidy level. Percent fruit set for self-pollinations averaged less than 1/4 of the cross-pollinations. The number of plump seeds per berry for self-pollinations averaged less than 1/3 that of the cross-pollinations. Self-pollination also gave a lower percent seed germination. The authors cited earlier studies showing that only 1 to a few well-developed seeds are necessary for normal fruit development in the greenhouse (Lyrene and Sherman, 1983). This implies that looking only at percent fruit set can give an inflated value for self-fertility. Vander Kloet and Lyrene recommended the number of seedlings produced per pollinated flower as a more sensitive indicator of fertility.

In general, researchers have used the term self-incompatibility to describe reduced fertility from self-pollination as compared to cross-pollination. More recently, a few researchers have chosen to restrict the term self-incompatibility to those factors preventing fertilization following pollination, so that no seed is set (Harrison et al., 1993; Krebs and Hancock, 1988, 1990). The literature shows that blueberry pollen tubes of both self and cross pollen grow at a similar rate, penetrate into the ovules and effect fertilization (El-Agamy et al., 1980; Garvey and Lyrene, 1987; Krebs and Hancock, 1988; Vander Kloet, 1991).

Krebs and Hancock (1990) hypothesized that variable self- and out-cross fertility in V. corymbosum is a consequence of early-acting inbreeding depression (seed abortion) and is regulated by the level of zygotic inbreeding generated by a given mating. It was found that fruit set (Harrison et al., 1993), seed set and seed abortion (Harrison et al., 1993; Krebs and Hancock, 1990) were reduced in self- and cross-pollinations as the inbreeding coefficient (F) of the parents or zygote increased. A positive correlation was found between self-fertility and cross-fertility; clones that were highly cross fertile tended to be highest in self-fertility. This suggested that the factors that affect self-fertility also

affect cross-fertility. These data were used to support the hypothesis that inbreeding depression rather than self-incompatibility reduces seedling production after self-pollination of blueberry (Harrison et al., 1993; Krebs and Hancock, 1990).

Vaccinium arboreum

Very little has been published about V. arboreum Marsh. This is likely due to the fact that the plant had little economic importance commercially or in home landscaping. Interestingly enough, it was grown in English gardens in the 18th century (Allgood, 1970).

Taxonomically, V. arboreum is in section Batodendron, genus Vaccinium, and family Ericaceae. It has 2 synonyms, Vaccinium diffusum Aiton (Camp, 1945) and Batodendron arboreum Marsh (Darrow, 1941). Common names include sparkleberry, farkleberry, winter-huckleberry, and tree-huckleberry (Camp, 1945; Ballinger et al., 1982). The name sparkleberry comes from the shiny black berries which have a tendency to hang on the tree most of the winter. Farkleberry is a corruption of the word sparkleberry. The name winter-huckleberry refers to the berries remaining on the tree through the winter. Its tree-like growth habit (when compared to huckleberries) led to the name tree-huckleberry (Ballinger et al., 1982; Camp, 1945).

Vaccinium arboreum is typically a small monopodial tree that can reach a height of 10 m with a trunk diameter of 35 cm. Twigs of the current season and trunk bark are reddish in color. The leaves are coriaceous, lustrous and somewhat persistent. The inflorescence is an elongated raceme. The long pedicels (8-12 mm) are subtended by leafy bracts. The white corolla is campanulate with 5 lobes, approximately 3-5 mm long. Anther sacs are awned. The berries are small, black, shiny and often described as dry, gritty and inedible. The fruit is produced on current season wood. The seeds are large, approximately 2 mm long (Ballinger et al., 1982; Galletta, 1975; Vander Kloet, 1988).

The range of the sparkleberry extends from southern Virginia to central Florida, west to eastern Texas, central Oklahoma and southeastern Missouri. It is rare and local in Illinois, Indiana, Kentucky, and Virginia (Vander Kloet, 1988). This species is usually found in sandy or rocky sites such as dry woodlands, thickets or clearings (Ballinger et al., 1982; Vander Kloet, 1988). Vaccinium arboreum is 1 of the few Ericaceous species that can tolerate calcareous soils (Ballinger et al., 1982). In Texas, V. arboreum has been found growing on sandy or sandy loam soils with a pH greater that 6.0. The organic matter content of these soils ranged from 0.1% to 4%, with a large

number of sparkleberry sites having soil organic matter under 1% (Stockton, 1976).

Research

Little research has been done with V. arboreum. Galletta and Fish (1971) examined the possibility of using V. arboreum as a rootstock for V. corymbosum. In 1963, they spring grafted 24 different highbush scions onto native V. arboreum plants that were either potted or in the woods. Successful takes for the grafts in the woods ranged from 30% to 95%. Grafts onto potted plants had a success rate that ranged from 14% to 100%. The potted V. arboreum plants proved difficult to transplant, and the technique was not adopted commercially. Some of the grafted scions were surviving in the woods with no care when checked in 1970.

Anthocyanins of sparkleberry fruit were identified by Ballinger et al. (1982). Separation and identification were accomplished using purified paper chromatography. The anthocycanins were identified as the 3-monogalactosides and 3-monoarabinosides of delphinidin, malvidin, peonidin, petunidin and cyanidin. Also present in small quantities were the 3-monoglucosides of delphinidin, petunidin, and peonidin. These anthocyanins are very similar to those reported for V. angustifolium (lowbush) and V. corymbosum (highbush).

Haywood (1993) studied seed germination over a 5 year period in the field. The purpose was to determine how long seeds remained viable in the field under 2 different conditions. Fruit that had not been cleaned, chemically treated or scarified were placed in fiberglass-screen pouches. Pouches were placed in 2 different types of sites in central Louisiana, under existing litter or buried 2.5 cm deep in a mineral soil. Each year for 5 years, 1 pouch at each site was removed and the number of newly germinated seeds counted. After counting, all the seeds in the pouch were discarded. The pouches recovered after 2 years had a germination rate in the mineral soil of 33% and 41% under the leaf litter. In the fifth year, 3% of the seeds in the recovered pouch, in the mineral soil, had germinated, and 5% of those under the leaf litter had germinated. This work indicated that V. arboreum seeds that have not germinated after 2 years are unlikely to do so.

Stockton (1976) also studied seed germination. Seeds were cleaned, dried and stored in a refrigerator for 7 months. They were planted at 3 different temperature ranges; 7-21 C, 10-24 C, and 21-32 C. Half of the seeds at each temperature range were placed under cool white light and half under red light. Germinated seeds were counted at 7 weeks. He found that seeds germinated better under cool temperatures than hotter temperatures and that seeds

germinated better under white light than red light. The best group had only a 10% germination rate.

Stockton (1976) also studied propagation from cuttings. Four levels of IBA were tested as possible rooting aids, 0 ppm, 10,000 ppm, 15,000 ppm and 20,000 ppm. These softwood cuttings failed to root after 60 days under mist.

Rhizome cuttings of various sizes were taken in October. The diameter ranged from 0.5 - 3 cm and the length from 10 - 30 cm. The cuttings were stuck in a peat-perlite mix. Orientation was vertical with the proximal end up and the distal end buried 1 inch deep. Shoots were produced within 21 days, but most of these died quickly. At the end of 3 months roots had formed. New shoots that were produced at this time were more successful. Stockton did not recommend this as a method of propagation. The time and labor needed to obtain the cuttings were excessive and there was also a problem with stock plant mortality.

Vaccinium arboreum has been used in the University of Florida blueberry breeding program (previously discussed) as a possible source of broader soil tolerance and for its wider, more open corolla. At the Horticultural Unit in Gainesville, Florida, it has been observed that V. arboreum seedlings show outstanding growth and survival on land where most Cyanococcus seedlings grow poorly due to low soil organic matter and irrigation with water high in

bicarbonates. Some of the tetraploid V. arboreum derivatives also seem to have this capability (Lyrene, 1993).

CHAPTER 3
MORPHOLOGICAL STUDY

Introduction

This study had 3 goals. The first was to compare the morphology of the F_1 hybrids with that of the parents, V. arboreum and V. darrowi. Intermediacy in morphology would indicate that the plants were actually the expected hybrids. The second goal was to examine evidence for the hypothesis that the MIKs are hybrids, and that highbush was the pollen parent of the MIKs. If the MIKs are intermediate in morphology between highbush and the F_1s, this would be evidence to support this hypothesis. The third goal was to show that V. arboreum genes are being transferred to subsequent generations when V. darrowi x V. arboreum F_1 hybrids are used as a genetic bridge, and that these genes are being expressed. Many characteristics of leaves, flowers, and berries were examined and measured in this study.

Materials and Methods

Except for the individuals of V. arboreum, all the plants used in this study were growing at the University of Florida Horticultural Unit in Gainesville, Florida. The V. arboreum plants were located at O'Leno State Park, 10 km north of High Springs, Florida. The V. corymbosum plants used were all cultivars or advanced selections of southern highbush blueberry.

Leaves

In the spring of 1994, leaves were collected from 20 genetically distinct plants of each of the following taxa: V. arboreum, V. darrowi, V. corymbosum, MIK, and the 16 V. darrowi x V. arboreum F_1s. Leaves were chosen at random from the outside canopy where they were exposed to full sun. Five leaves were collected from each clone, pressed and stored for later examination.

The leaf characters measured were: length, width (at widest point), distance from base to widest point, and leaf shape (length divided by the distance from the base to the widest point). Stalked glands were scored as present or absent. Pubescence was scored as none present, low, medium or high. Marginal bump glands were scored as exserted or sunken. All data were collected using a dissecting scope.

Flowers

Flowers were collected in the spring of 1994. Highbush and MIK flowers were collected during the first week of March. Vaccinium darrowi and V. arboreum flowers were collected on April 1. Five inflorescences were collected from each of 20 different genotypes. Flowers from 14 of the F_1s were harvested on April 6. F_1 clone 85-128 was harvested the following year, April 4, 1995. No flowers were produced either year by F_1 clone 85-136 . Inflorescences were preserved in 70% ethanol for later examination.

The characteristics measured were: corolla length, corolla width, corolla aperture, pedicel length, peduncle length, bracteole length and bracteole width. Anther awns, filament curvature and bracteoles were scored as absent or present. Measurements and observations were made using a dissecting scope.

Berries

Fifty berries were harvested from each of 20 different genotypes of highbush, V. darrowi, V. arboreum, and the MIKs. Only 7 F_1s produced enough berries for all or part of this study. They were 85-125, 85-126, 85-127, 85-129, 85-130, 85-131 and 85-134. The V. darrowi berries were

harvested from May 12, 1994 through May 30, 1994. Highbush berries were picked on April 27, 1994. Vaccinium arboreum berries were all harvested on Oct. 19, 1993. Berries from the MIKs were harvested April 26, 1994 through May 23, 1994. Four F_1s were harvested June 1, 1994 through July 26, 1994. The other 3 were harvested between July 7 and July 25, 1995.

The characteristics measured were berry weight and large-seed weight. Thirty berries were weighed per clone. Berries to be weighed were selected randomly from the 50 that were harvested and were divided into 2 replications of 15. After the berries were weighed, the seeds were removed using a food blender. The seeds were dried overnight on a laboratory bench and weighed the next morning. Vaccinium arboreum berries contain sclerids that aggregate into large clumps around the seeds. These were removed with a #25 sieve (0.7128 mm) before weighing. The other taxa contain sclerids, but they do not aggregate into clumps and are washed away during seed extraction. The 30 largest seeds were then removed, divided into 2 replications of 15, and weighed for each clone.

Results and Discussion

Leaves

The length and width of the F_1 leaves were intermediate between those of the 2 parents, V. darrowi (small) and V. arboreum (large) (Table 1 and Figure 1 and 2). The mean leaf lengths for the 3 taxa differed significantly (P=0.01). The upper range of the F_1 hybrid population overlapped slightly with the lower range of the V. arboreum population (Appendix Table 36). Mean leaf width also differed significantly among the 3 taxa (P=0.01). The population ranges of the 3 overlapped slightly (Appendix Table 27). Although mean leaf length of the F_1s was almost exactly midway between the 2 parents, mean leaf width of the F_1s was much closer to V. darrowi than to V. arboreum.

MIK leaves were intermediate in length and width between those of the F_1s and V. corymbosum (hypothetical pollen parent) (Table 1 and Figure 3). In both characters, means of the MIKs were closer to V. corymbosum than to the F_1s. The ranges of all 3 taxa overlapped somewhat.

The intermediacy of the F_1s and the MIKs between their hypothetical parents in leaf length and width was consistent with their proposed pedigrees.

Table 1. Leaf characteristics of 5 taxa: V. darrowi (Dar), V. arboreum (Arb), F_1 (V. darrowi x V. arboreum hybrid), southern highbush (HB) and MIK (open-pollinated progeny of the F_1 hybrids).

	Taxa means[z]				
Characters	Dar	Arb	F_1	HB	MIK
Length (mm)	10.9d[y]	36.3b	22.6c	45.1a	38.6b
Width (mm)	5.2d	22.5a	9.6c	24.4a	18.4b
Distance from base to widest point (mm)	6.4	20.8	13.3	22.1	21.6
Leaf shape[x]	1.71b	1.76b	1.72b	2.05a	1.79b
Stalked glands (percent with)	0.0	100.0	50.0	0.0	20.0
Pubescence					
-percent with none	25.0	0.0	0.0	80.0	5.0
-percent low	40.0	95.0	88.0	20.0	90.0
-percent medium	35.0	5.0	0.0	0.0	5.0
-percent high	0.0	0.0	12.0	0.0	0.0
Margin glands					
-percent with none	0.0	0.0	0.0	75.0	0.0
-percent with sunken	95.0	0.0	6.0	20.0	50.0
-percent with exserted	5.0	100.0	50.0	0.0	35.0
-percent with both	0.0	0.0	44.0	5.0	15.0

[z]Each mean is the average of 20 genetically distinct plants with 5 replications (leaves) per plant, except the F_1 hybrids which had 16 plants.

[y]Mean separation within rows by LSMeans with a Tukey-Kramer adjustment on log transformed data. Means followed by a common letter do not differ significantly at the P = 0.01 level. Actual means are presented here.

[x]Leaf shape was calculated by dividing length by distance from base to widest point.

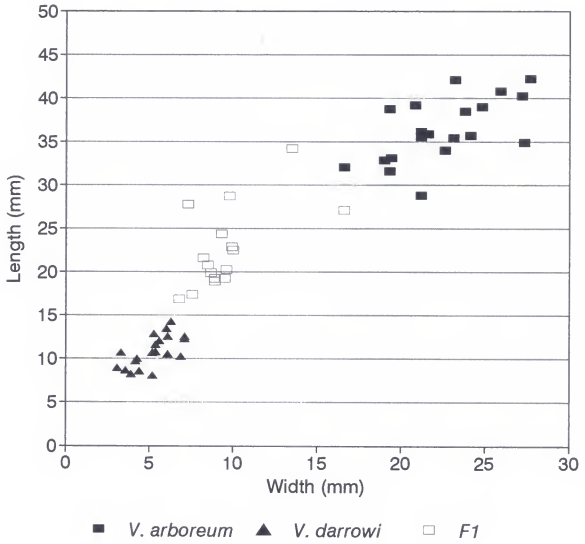

Figure 1. Leaf length and width of V. darrowi, V. arboreum and the F_1s (V. darrowi x V. arboreum hybrids). Each point represents the mean of 5 replications for 1 plant.

Figure 2. Examples of leaves from V. arboreum (left), V. darrowi (right) and their F_1 hybrid (middle).

Leaf shape population ranges for all 5 taxa were overlapping (Figure 4 and Appendix Table 39). The leaf shape mean for highbush was significantly (P=0.01) higher than for the other 4 taxa (Table 1). However, the overlap in the ranges of highbush and the other taxa made this characteristic a poor tool for distinguishing among them.

Stalked glands are found on the abaxial surface of the leaf blade of some Vaccinium species. These glands were found on all of the V. arboreum leaves examined. None of the V. darrowi leaves included in this study had stalked glands. In a survey of V. darrowi in Florida, however, Lyrene (1986) found that 9 out of 15 clones surveyed had stalked glands. 'Johnblue', a V. darrowi clone that was crossed with V. arboreum to produce some of the hybrids used in this study, was examined and found to have stalked glands. Of the 6 F_1s with 'Johnblue' as a parent, 2 did not have stalked glands and 4 had them. Overall, 50% of the F_1s were found to have stalked glands (Table 1). None of the highbush leaves examined had stalked glands. In the MIK population, 20% of the clones exhibited them.

From the data presented in Table 1, it appeared that the stalked gland character could help answer the questions this study posed. However, due to the presence of stalked glands in 'Johnblue' (not included in the original study)

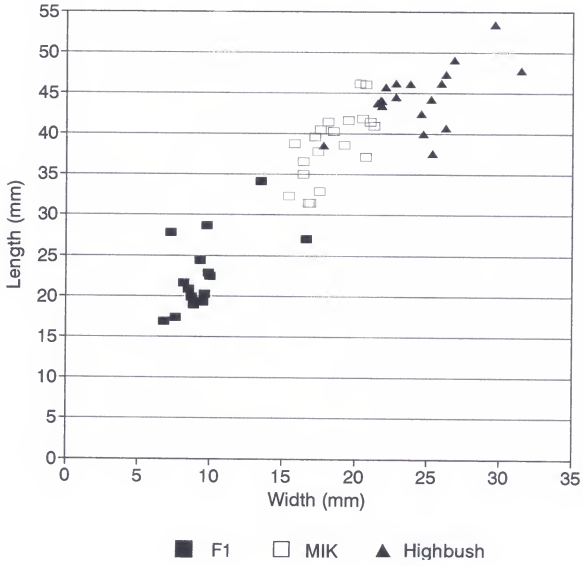

Figure 3. Leaf length and width of highbush, MIKs (open-pollinated progeny of the F_1 hybrids) and F_1s (*V. darrowi* x *V. arboreum* hybrids). Each point represents the mean of 5 replications per plant.

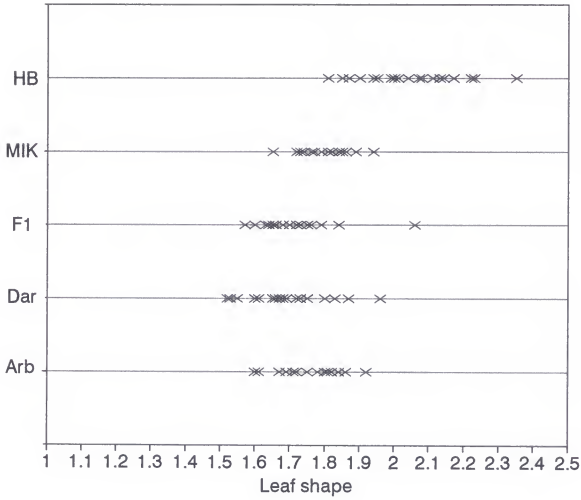

Figure 4. Ranges for leaf shape in V. arboreum (Arb), V. darrowi (Dar), F_1 (V. darrowi x V. arboreum hybrids), MIK (open-pollinated progeny of F_1 hybrids) and southern highbush (HB) taxa. Each point represents the mean of 5 replications for 1 plant.

and Lyrene's work in V. darrowi, this character could not give conclusive evidence.

Abaxial leaf pubescence was found on all V. arboreum and F_1 plants, 75% of the V. darrowi plants, 20% of the highbush, and 95% of the MIKs (Table 1). Most plants, within all 5 taxa that had pubescence, were scored as low. Twelve percent of the F_1 plants were highly pubescent. This was the only taxon that showed a high level of pubescence. Vaccinium darrowi and highbush were the only taxa in which some plants had no pubescence, 25% and 80% respectively. Pubescence provided very little information to answer the questions this study posed.

The marginal glands on the basal half of V. arboreum leaves can be easily seen with a 10x field lens. On most V. arboreum leaves the glands are present along the entire margin. There are no reports of marginal leaf glands in V. darrowi or highbush (Goffery, 1988 and Vander Kloet, 1988). All the taxa in this survey exhibited marginal glands, at least in some individual plants. Seventy-five percent of the highbush plants had no glands. Of the 25% that had glands, most of the glands were sunken into the margin. All the plants in the other 4 taxa had marginal glands. In V. darrowi, 95% of the plants had glands that were sunken as the highbush glands were. All of the V. arboreum plants and 5% of the V. darrowi plants had glands that were exserted

from the margin. The fact that they were exserted made the glands much more obvious to casual observation. Fifty percent of the F_1 plants had glands that were exerted as on the V. arboreum parent, 6% of the glands were sunken and 44% contained both types. All of the MIKs had marginal leaf glands. Thirty-five percent of the plants had exserted glands, 50% had sunken glands and 15% displayed both types. The large percentage of exserted marginal glands in the MIKs, compared to 5% in V. darrowi and 0 in highbush, indicated the presence and expression of V. arboreum genes.

Flowers

Corolla size of the F_1s was intermediate between their parents, V. darrowi and V. arboreum (Figures 5,6 and Table 2). Mean corolla length differed significantly (P=0.01) for these 3 taxa. Surprisingly, the mean corolla length of the F_1s exceeded both parents. The mean corolla width of the F_1 population was significantly different (P=0.01) from V. arboreum, but not from V. darrowi.

The MIK population was also intermediate in corolla size between the F_1s and highbush (Figure 7 and Table 2). The mean corolla length of the MIKs was significantly different (P=0.01) from that of their F_1 parents, but not

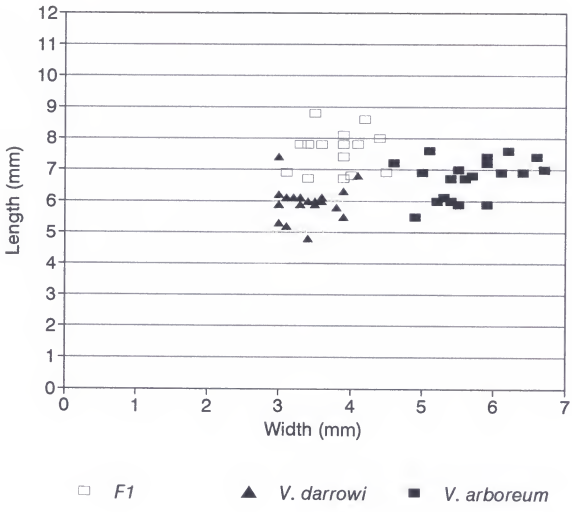

Figure 5. Flower size for V. darrowi, V. arboreum, and the F_1 (V. darrowi x V. arboreum hybrids). Each point represents the mean of 5 replications for 1 plant.

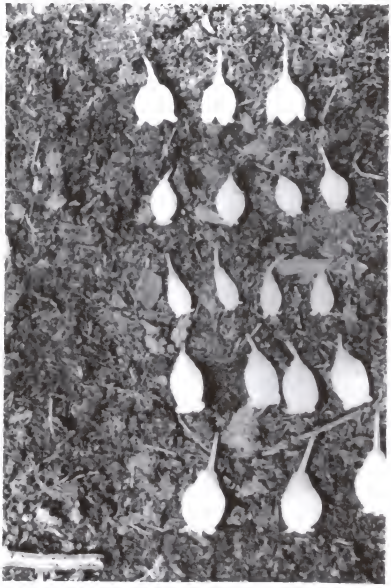

Figure 6. Flower morphology of V. arboreum (top), V. darrowi (2nd row), their F1 hybrid (3rd row), and MIKs - open-pollinated progeny of F1 hybrids (bottom 2 rows).

Table 2. Floral characteristics of 5 taxa: V. darrowi (Dar), V. arboreum (Arb), F_1 (V. darrowi x V. arboreum hybrids), southern highbush (HB), and MIK (open-pollinated progeny of the F_1 hybrids).

	Taxa means[z]				
Character	Dar	Arb	F_1	HB	MIK
Corolla length (mm)	6.0d[y]	6.7c	7.6b	10.3a	9.9a
Corolla width (mm)	3.4c	5.6b	3.8c	6.2a	5.4b
Corolla aperture (mm)	1.7d	5.0a	2.3c	3.2b	3.1b
Pedicel length (mm)	4.3c	11.6a	6.7b	4.2c	5.8b
Peduncle length (mm)	4.1[x]d	44.3a	15.8b	9.1c	11.3bc
Bracteole length (mm)	1.7[xv]c[v]	1.7[u]c	2.7[t]b	3.8a	3.5a
Bracteole width (mm)	1.1[xv]c[s]	0.3[u]d	0.9[t]c	2.2a	1.5b
Anther awns					
(percent with)	0.0	100.0	93.0	0.0	60.0
(percent with nubs)	25.0	0.0	7.0	20.0	35.0
Filament curvature					
(percent with)	100.0	100.0	87.0	100.0	65.0
Persistent bracteoles					
(percent with)	70.0	30.0	67.0	100.0	100.0

[z]Each mean is the average of 20 genetically distinct plants with 5 replications per plant, except the F_1 hybrids which had 15 plants.

[y]Mean separation within rows by LSMean with a Tukey-Kramer adjustment, P = 0.01.

[z]Mean separation was performed on log transformed data. Actual means are presented here.

[x]Average of 14 plants

[v]P=0.04

[u]Average of 4 plants

[t]Average of 9 plants

[s]P=0.02

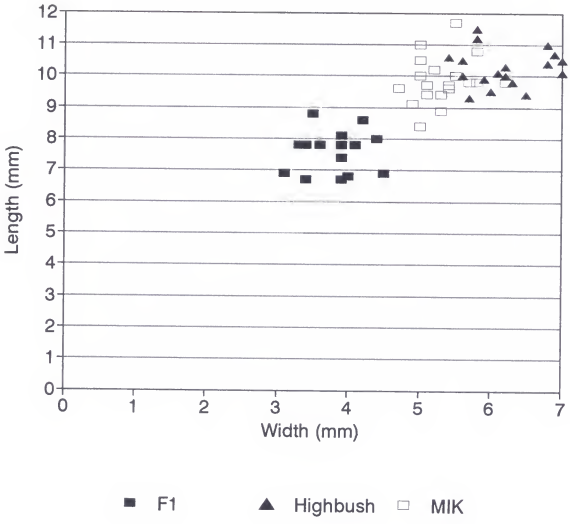

Figure 7. Corolla size of F_1 (V. darrowi x V. arboreum hybrids), MIKs (open-pollinated progeny of the F_1 hybrids) and highbush. Each point represents the mean of 5 replications for 1 plant.

from highbush. The population ranges of the MIKs and highbush were almost identical (Appendix Table 40). The means for corolla width were significantly different (P=0.01) for all 3 taxa.

The mean corolla size of the F_1 is a good indicator that the F_1s are actually hybrids of V. darrowi and V. arboreum. Despite the lack of significance for the difference in mean corolla width between the F_1 and V. darrowi populations, the data suggest 3 distinct populations (Figure 5). These data also supported the theory that highbush was the pollen parent of the MIKs. The MIKs and highbush did not differ significantly in mean corolla length, but again, 3 distinct population groupings, with the MIKs intermediate, were evident (Figure 7).

The mean corolla aperture for V. darrowi, V. arboreum, and the F_1s were significantly different (P=0.01) from each other (Table 2), with V. arboreum the largest and V. darrowi the smallest. The ranges (Figure 8 and Appendix Table 42) of V. darrowi and the F_1 overlapped considerably. The F_1s were significantly smaller (P=0.01) than the MIKs, but the MIKs and highbush did not differ significantly. The ranges of the MIKs and highbush were almost identical and overlapped with the upper range of the F_1s. Corolla aperture again contributed evidence to support the hybrid

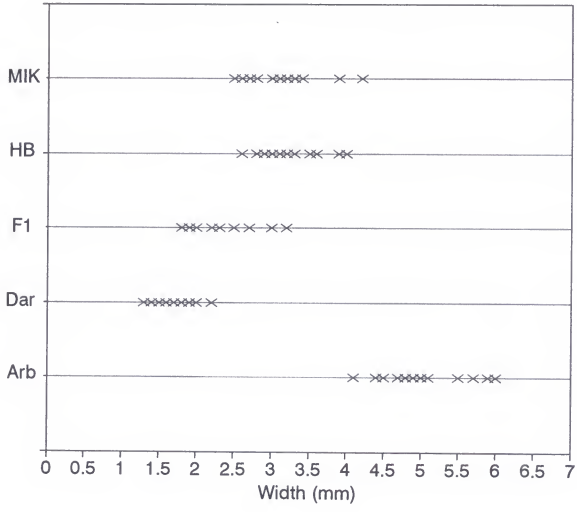

Figure 8. Flower aperture for V. arboreum (Arb), V. darrowi (Dar), F_1 (V. darrowi x V. arboreum hybrids), MIKs (open-pollinated progeny of F1 hybrids) and southern highbush (HB). Each point represents the mean of 5 replications for 1 plant.

nature of the F_1s. Mean aperture for the MIKs was essentially the same as for highbush. While, the MIKs were not intermediate between these 2 taxa, the data did not rule out highbush as pollen parent.

Long pedicels (secondary axis of the inflorescence) are characteristic of V. arboreum (Figure 11). The mean pedicel length of V. arboreum was more than twice that of V. darrowi (Table 2). The mean of the F_1s was between the parental means. It was significantly different (P=0.01) from both parents, but was much closer to that of V. darrowi. The range of the F_1s extended into the range of V. arboreum and covered that of V. darrowi (Figure 9 and Appendix Table 43). This provided more evidence for the F_1's hybridity.

The mean pedicel length of the MIKs (Table 2 and Figure 12) was significantly greater (P=0.01) than that of highbush, but not different from the F_1s. The ranges of the 3 taxa overlapped considerably. However, the clustering of the individual means indicated that the MIKs were intermediate with respect to the other 2. Highbush did not differ significantly (P=0.01) from V. darrowi.

Long peduncles (primary axis of the inflorescence) are another characteristic of V. arboreum (Figure 11). Mean peduncle length for V. darrowi, V. arboreum, and the F_1s were statistically different (P=0.01) (Table 2). The mean for V. arboreum was 10x that of V. darrowi. The range of

the F_1s covers the distance between the upper and lower ranges of the other 2 taxa (Figure 10 and Appendix Table 44). This was more evidence for the hybrid nature of the F_1s.

There was no significant difference (P=0.01) (Table 2) between the MIKs and the F_1s for mean peduncle length. There also was no significant difference between the MIKs and highbush. However, the ranges of the 3 taxa overlapped in a manner that indicated intermediacy of the MIKs between the other 2 taxa (Figure 10 and Appendix Table 44).

The bracteoles (2 small bractlets located on the pedicel) in some blueberry taxa are quickly deciduous, falling off soon after the flowers open. In V. arboreum only 30% of the plants had bracteoles (Table 2) that remained on the inflorescences at the time of examination. Seventy percent of the V. darrowi plants had inflorescences that had retained their bracteoles. The retention of bracteoles in the F_1s was very close to V. darrowi at 67%. All of the MIK and highbush plants examined had inflorescences that had retained their bracteoles. The presence of bracteoles in the hybrids was essentially the same as for the Cyanococcus parent.

The mean bracteole length of V. arboreum and V. darrowi were identical (Table 2), as were their ranges (Figure 13 and Appendix Table 45). The mean bracteole length of the

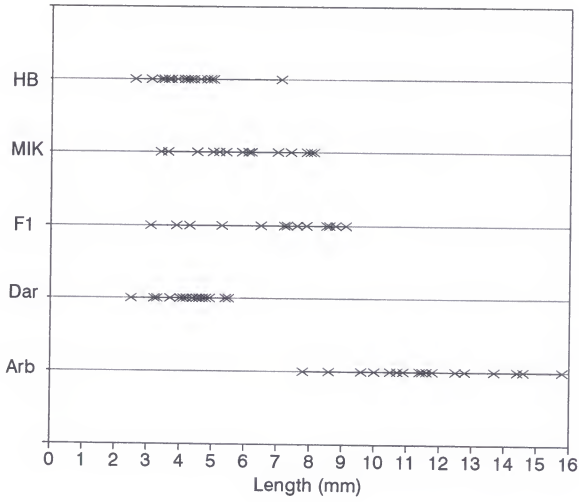

Figure 9. Pedicel length for V. arboreum (Arb), V. darrowi (Dar), F_1 (V. darrowi x V. arboreum hybrids), MIKs (open-pollinated progeny of F_1 hybrids), and southern highbush (HB). Each point represents the mean of 5 replications for 1 plant.

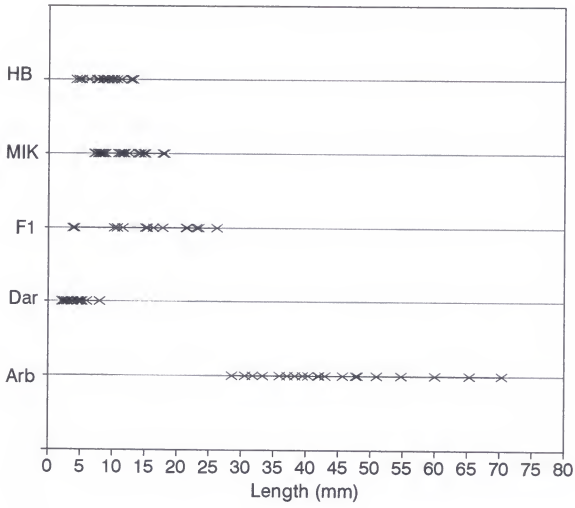

Figure 10. Peduncle length for V. arboreum (Arb), V. darrowi (Dar), F_1 (V. darrowi x V. arboreum hybrids), MIKs (open-pollinated progeny of the F_1 hybrids) and southern highbush (HB) taxa. Each point represents the mean of 5 replications for 1 plant.

Figure 11. Inflorescence morphology of V. arboreum (left), highbush (right), and the F_1 hybrid (middle). Note the characteristically long pedicels and peduncles of V. arboreum.

Figure 12. Fruiting MIK (open-pollinated progeny of F_1 progeny) showing long pedicels and peduncles.

F_1s was significantly different (P=0.04) from its parents and exceeded both of them by approximately 33%. The lower range of the F_1s barely overlapped the upper range of both parents. This is another example of transgressive segregation in the F_1s, corolla length being the other.

There was a significant difference (P=0.02) for mean bracteole width between V. arboreum and V. darrowi. Vaccinium darrowi was approximately 3.5x wider than V. arboreum. The mean bracteole width of the F_1s was slightly smaller but not significantly different from V. darrowi (Table 2 , Figure 13, and Appendix Table 46).

The mean bracteole length of the MIKs was significantly greater (P=0.04) than that of the F_1 parent (Table 2). There was no significant difference between the MIKs and highbush, although the mean bracteole length of the MIKs was slightly smaller. The upper range of the F_1s overlapped with the lower range of highbush (Figure 14 and Appendix Table 45). The range of the MIKs for mean bracteole length spanned almost the entire combined range of highbush and the F_1s.

The mean bracteole width of the MIKs was intermediate between and was significantly different (P=0.02) from highbush and the F_1s (Table 2). The population range of the MIKs overlapped that of the highbush and F_1 populations (Figure 14 and Appendix Table 46).

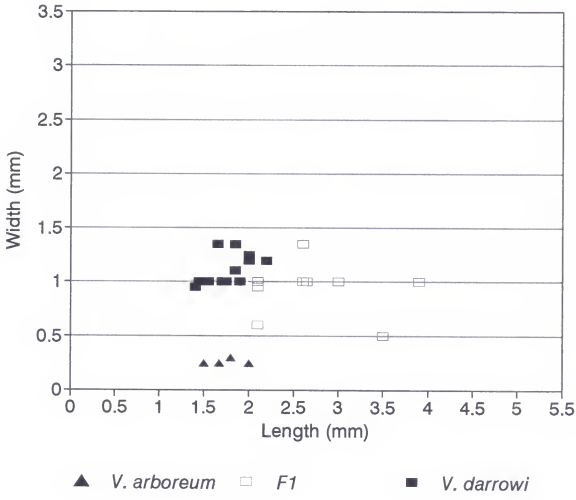

Figure 13. Bracteole length and width for V. darrowi, V. arboreum, and the F_1s (V. darrowi x V. arboreum hybrids). Each point represents the mean of 5 replications for 1 plant.

Bracteole length and width added further evidence regarding the origin and hybrid nature of the F_1 and MIKs. The F_1 bracteoles retained the lanceolate shape of their V. arboreum parent. Although not as pronounced, the MIK bracteoles also had a lanceolate shape. The shape of highbush and V. darrowi bracteoles were more ovate (wider at the base) than lanceolate.

There was not enough variation among taxa to make filament curvature a useful character for answering the questions this study posed (Table 2). Surprisingly, the only taxa with some straight filaments were the 2 hybrid taxa.

All V. arboreum anthers had awns (Table 2). None of the V. darrowi had awns, but 25% of the plants did have anther nubs, a small protuberance where the anther awn would have been. Ninety-three percent of the F_1 hybrids had anther awns, most of which were about half the length of those found in V. arboreum. The remaining 7% had nubs as found in V. darrowi. Anther awns are not found in V. darrowi; therefore, this was an excellent trait for hybrid confirmation.

None of the highbush plants had fully developed anther awns. Nubs were found in 20% of the plants examined. Sixty percent of the MIKs had anther awns and 35% had nubs. The presence of anther awns in the MIKs was evidence that V.

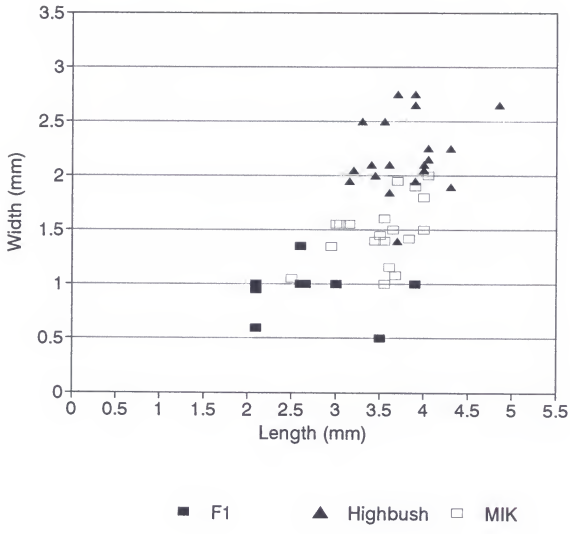

Figure 14. Bracteole length and width of the F_1s (V. darrowi x V. arboreum hybrids), highbush(HB), and MIKs (open-pollinated progeny of the F_1 hybrids) and. Each point represents the mean of 5 replications for 1 plant.

arboreum genes had been passed to the next generation and were being expressed.

Berries

Vaccinium arboreum berries averaged slightly, but significantly (P=0.01), heavier than the berries of V. darrowi (Figure 15 and Table 3). The mean berry weight of the F_1s was identical to V. darrowi. Berry weight for the MIKs was intermediate and significantly different (P=0.01) from the F_1s and highbush. The intermediate nature of the hybrids was further supported by these data.

Large seeds were characteristic of V. arboreum. Visually, they were conspicuously larger than the seeds of cultivated blueberries. The largest seeds of V. arboreum were significantly heavier (P=0.01) than those of V. darrowi (Table 3 and Figure 16). The mean weights of the largest seeds of the F_1s was not significantly different (P=0.01) from the V. arboreum parent. This is a character that again shows the hybrid nature of the F_1s. The difference in the seed size is sufficient to permit rapid identification of hybrids in the field.

The mean weight of the largest seeds of highbush were significantly less (P=0.01) than for the F_1s (Table 3). Seeds of the MIKs were significantly larger than those of the highbush, but not as large as the seeds of the F_1s.

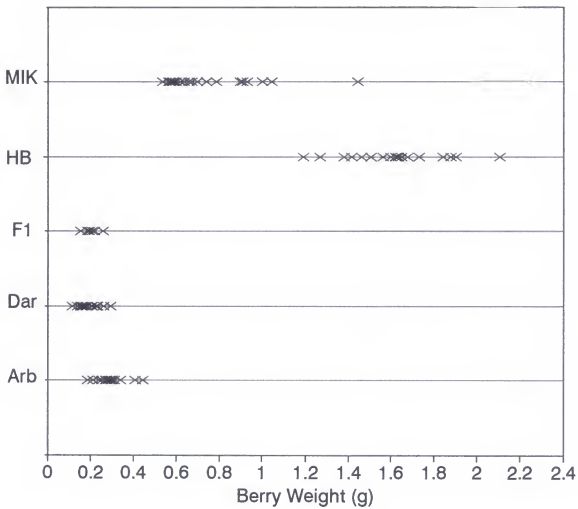

Figure 15. Berry weight for V. arboreum (Arb), V. darrowi (Dar), F_1 (V. darrowi x V. arboreum hybrids), MIK (open-pollinated progeny of F_1 hybrids), and southern highbush (HB). Each point represents the mean of 5 replications for 1 plant.

Table 3. Berry characteristics of 5 taxa. Each mean is the average of 20 open-pollinated genetically distinct plants except in the case of the F_1 hybrids, where there were not 20 fruitful plants available.

Taxa	Berry weight[z] (g)	Mean of 15 large seeds[y] (mg)
V. darrowi	0.20 d[x]	7.25 d
V. arboreum	0.28 c	20.25 a
Highbush	1.65 a	10.40 c
F_1[w]	0.20[v] d	19.60[u] ab
MIK[t]	0.77 b	14.80 b

[z]Average of 2 replications from each plant, each composed of 15 berries.
[y]Average of 2 replications from each plant, each composed of 15 large seeds.
[x]Means within columns separated by LSMeans with a Tukey-Kramer adjustment on transformed data. Actual means are presented here.
[w]V. darrowi x V. arboreum hybrids
[v]Average of 7 F_1s, 5 plants of which had 2 replications each, 2 plants of which had only 1 replication.
[u]Average of 3 F_1s, 2 replications for each plant
[t]Open-pollinated progeny of F_1 hybrids

Seed weight furnished further evidence that V. arboreum genes have been being passed to subsequent generations and are being expressed. The large seed weight could only have come from V. arboreum.

Plant Architecture

The plant architecture of the hybrids was intermediate between their parents. Vaccinium darrowi is a shrub that reaches a height of 1 to 2 m (Lyrene, 1986) when grown in

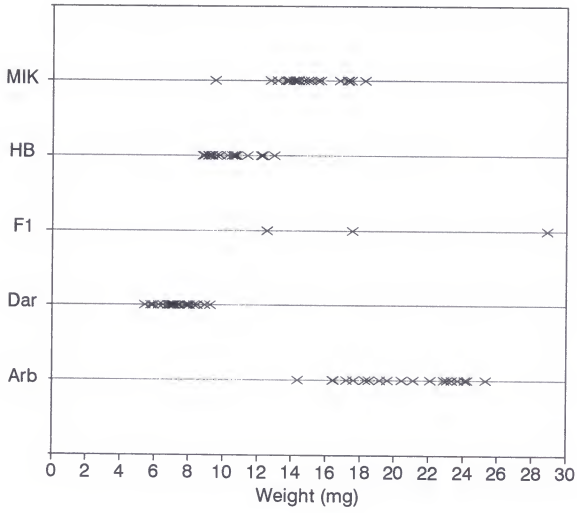

Figure 16. Mean weights of 15-seed samples of large seeds for V. arboreum (Arb), V. darrowi (Dar), F_1 (V. darrowi x V. arboreum hybrids), MIKs (open-pollinated progeny of F_1 hybrids), and southern highbush (HB). Each point represents the mean of 2 replications for 1 plant.

partial shade, but would normally attain a height of only about 1 m under the conditions that prevailed where they were planted at the Horticultural Unit. Vaccinium arboreum is a small tree, frequently monopodial, attaining a height of up to 10 m (Godfrey, 1988 and Vander Kloet, 1988). The F_1s are large shrubs that ranged in height from 1.6 m to 3.0 m after 13 years in the field. The F_1s have the suckering and twiggy habit of their V. darrowi parent. Highbush ranges in height from 1-3(4) m (Camp, 1945). The MIKs are very similar in height and form to their hypothetical highbush parent.

Summary

Five of the characters examined gave no useful information relative to the questions asked in this study. These characters were leaf shape, stalked glands, pubescence, presence of bracteoles, and filament curvature.

The F_1s were intermediate in morphology between the parents, V. arboreum and V. darrowi, for most of the traits analyzed. For certain characters the F_1s were identical to 1 of the parents. For only 2 characters did the F_1 population mean fall outside the ranges of the parents. These characters were mean corolla length and mean bracteole length. In both of these the F_1s exceeded both parents.

The means for the MIKs were intermediate between the means of their hypothesized parents, F_1s and highbush, in 7 out of 15 characters. For 5 additional characters, the population range of the MIKs was very close to, though not outside the population ranges, of 1 or the other parent.

Four of the characters examined clearly indicated that V. arboreum genes have been transferred to subsequent generations and are being expressed. These were marginal glands, anther awns, bracteole shape, and large seed weight.

CHAPTER 4
FERTILITY OF V. DARROWI x V. ARBOREUM HYBRIDS AND INTROGRESSION LINES

Introduction

Crossing Studies

Crosses were made in the greenhouse using the F_1s, MIKs and MIK derivatives in 1992, 1993, and 1994. There was a wide range of female fertility in the MIK populations; so only the most fertile plants were selected for this study. Controlled crosses were also made using V. corymbosum, V. darrowi, and V. arboreum.

The purpose of making these crosses was multifold. First, they were used to estimate the female fertility of various clones. It was difficult to make this assessment in the field where plants were exposed to freeze, insect and animal damage. Second, various crosses with MIKs and F_1s were made in an effort to determine the male parent of the original MIKs. Finally, the crosses permitted a search for anomalies that can appear in the progeny of a wide cross e.g. self-compatibility. This will be discussed in Chapter 5.

Cytogenetics

Chromosome counts were made to confirm the suspected ploidy level of the F_1s and the MIKs. The F_1s were the progeny of 2 diploids and their ploidy level was expected to be diploid also. The original MIKs were the open-pollinated progeny of the F_1s. Many of their morphological features indicated that they were tetraploid, i.e. much larger flowers, fruit and leaves than the F_1s. The dramatic increase in the fertility of many MIKs over that of the F_1 parents also suggested that they might be tetraploid.

Total Seed Weight

Data for total seed weight of 50 berries were collected in the field for all 5 taxa involved in this study. The clones used in this study were the same ones used in the morphological study previously discussed (Chapter 3).

Mean seed weight per berry is 1 method of assessing female fertility in blueberries. The analysis of these data assumes that plenty of viable, genetically compatible pollen was available for fertilization. Because these plants were surrounded by other clones of the same and different taxa, this was a valid assumption.

Pollen

Pollen stainability and amount of pollen shed were used to assess male fertility for the F_1s, MIKs and MIK derivatives. Southern highbush, V. arboreum, and V. darrowi were also measured to provide a basis for comparison.

Materials and Methods

Greenhouse Crossing Studies

Plants used in greenhouse crosses were dug from the Horticultural Unit at the University of Florida, Gainesville in December 1991, 1992, and 1993. These plants included southern highbush, V. darrowi, V. arboreum, F_1 (V. darrowi x V. arboreum hybrids), MIKs, and MIK derivatives. The MIKs and MIK derivatives were ones that had been selected to be parents based on having had high fruit set in seedling nurseries in the field the previous spring. The plants were potted in peat and placed in a cooler kept at approximately 5C. Plants remained there for a minimum of 1 month to satisfy their chilling requirements. This facilitated synchronous blooming, and made it possible to cross plants with different chilling requirements. Following the chilling period, plants were placed in a bee-proof greenhouse where the temperature was maintained between 5C and 27C.

Some plants were large enough to use for 2 or 3 types of crosses. These were divided into sections with approximately equal numbers of flower buds per section. Crosses were randomly assigned to sections. In some cases, it was necessary to use more than 1 ramet of a clone for multiple crosses. On the day of pollination, the corolla and anthers were removed. Freshly gathered pollen was applied to the sigma from the thumbnail. The number of flowers pollinated varied for each cross. Before pollination, the male parent was checked for pollen stainability. This was to ensure that low fruit set would not be due to inviable pollen. Due to pollen stainability counts of 0 for the F_1s used in this study, no $F_1 \times F_1$ crosses were made.

Ripe berries were harvested daily and stored at 5C if necessary. Data were collected for various crosses on: fruit set, total seed weight, large seed weight, berry weight, number of plump seeds, and number of seeds germinated.

After harvest was complete, the berries were weighed. In 1993 and 1994, the number of plump seeds per berry were counted. This was done by squashing individual berries on paper towels. The seeds were left to dry overnight on a laboratory bench top. The next day, plump seeds were separated from non-plump seeds and counted. This was done

for 30 berries from each cross. The remaining berries were also squashed individually to see if plump seeds were present, but seeds were not counted. Large, plump seeds were separated from other seeds for each cross and weighed as previously described (Chapter 3). In 1992, seeds were removed using a food blender. Seeds were dried overnight on a paper towel in the laboratory. All seeds were weighed and stored.

Seeds were planted in December, 1992, 1993 and 1994. The night before planting, seeds were soaked in 10.4 mM gibberellic acid for approximately 12 hours (Dweikat and Lyrene, 1989a). Seeds from each cross were sown in a pot of peat. Top diameter of the pots was 10 cm. The pots were placed in a greenhouse where the temperature was maintained between 5C and 27C. A mist system was used for watering. In February, the seedlings were counted and a maximum of 96 from each cross were transferred to flats. The flats were maintained in a greenhouse until early May, at this time the seedlings were transplanted into a high-density nursery at the Horticultural Unit at the University of Florida, Gainesville, Florida.

Cytogenetics

Vegetative apical meristem tips are the best material for examination of somatic chromosomes in blueberry.

Actively growing shoot tips were collected and fixed in 3 absolute alcohol:1 glacial acetic acid for 24 hours. The fixative was then replaced with 70% ethanol and shoot tips were stored at 5C.

Prior to examination, the ethanol was removed by soaking the shoot tips in water. At this time each shoot tip was trimmed closely to the meristem. Shoot tips were then soaked in 1 N HCl for 30 minutes after which they were rinsed in water. Another soak followed in a pectinase-cellulase solution for approximately 24 hours. The solution consisted of 40 units pectinase and 40 units cellulase in 1 ml of water. At the end of the soaking period, the shoot tips were blotted on a paper towel, rinsed in water and blotted again. Shoot tips were then transferred to a drop of 1% aceto-carmine stain (1 g carmine boiled in 100 ml 45% acetic acid) for 1 minute and blotted. Tissue was transferred to a microscope slide and a small drop of aceto-carmine was added. The tissue was macerated with an iron needle (iron aids the staining process). The debris was removed and a coverslip applied. The slide was heated gently over a flame for approximately 1 minute. The coverslip was tapped with a pencil eraser and pressure was applied with a paper towel between the slide and the thumb. To destain, 45% acetic acid was introduced under the cover slip, the slide was heated again, and pressed to expel the

dye. Destaining was repeated 3 times. A phase contrast microscope (Leitz) was used to observe the chromosomes at a 1000x magnification.

Total Seed Weight

Fifty berries resulting from open-pollination were randomly harvested from each of 20 different genotypes of each of the following taxa: highbush, V. darrowi, V. arboreum, and the MIKs. All plants, except V. arboreum, were located at the Horticultural Unit, University of Florida at Gainesville, Florida. The V. arboreum plants were located at O'Leno State Park, Florida. Only 4 F_1s produced enough berries for this study. The V. darrowi berries were harvested from May 12, 1994 through May 30, 1995. Highbush berries were picked on April 27, 1994. Vaccinium arboreum berries were all harvested on Oct. 19, 1994. The F_1s were harvested June 1, 1994 through July 26, 1994.

The character measured in this study was total seed weight of 50 berries. The seeds were removed from the berries using a food blender. The seeds were dried overnight on a laboratory bench and weighed the next morning. The V. arboreum berries contained sclerids that aggregated into large clumps. These were removed with a #25 sieve (0.7128 mm) before the seeds were weighed.

Pollen

Flowers were collected to use in pollen studies from plants located in the high-density nursery. These included MIKs and MIK derivatives. F_1s were also collected. Collections took place February through April, 1992. Flowers for pollen were collected along with flowers for the morphology study for highbush, MIKs, V. darrowi, and V. arboreum. Flowers were stored in open glass vials until examination.

To determine if pollen was viable, it was stained with 2% aceto-carmine glycerine jelly (Radford et al., 1977) This medium is liquid in bulk at room temperature, but on a slide becomes a jelly. The viscous nature of the jelly prevents the lighter, empty pollen grains from being pushed to the outer edges (often a problem with aqueous solutions) when the cover slip is applied. This allows for a more random distribution of normal and empty pollen grains. Pollen staining was used as a measure of male fertility.

Pollen was removed from the flowers by gently twirling the flower over a drop of the 2% aceto-carmine glycerin jelly on a slide. Pollen from 2 flowers was mixed together for each clone. Each genotype was rated for the amount of pollen shed using an 6-point scale. The scale ranged from 0-5, with 0 representing no pollen and 5 representing a

copious amount of pollen. The slides were stored in the refrigerator for 48 hours before microscopic examination. This allowed time for the pollen grains to absorb the dye. To prevent desiccation, the slides were placed in a metal pan with a damp paper towel inside a sealed plastic bag.

Pollen was examined at 400x on a Leitz light field microscope. Pollen grains that were plump and well stained were scored as viable. Grains that were granular, not stained, poorly stained, or shrunken were scored as inviable. Two-hundred grains per clone were scored. If a tetrad was positioned so that 1 of the grains was not clearly visible, the grain was not scored.

Results and Discussion

Crossing Studies

Statistics

The means for the data collected from the controlled crosses were separated using Least Square Means with a Tukey-Kramer adjustment. This is the most appropriate mean separation technique to use when there is a difference in the number of plants used per treatment (Hochberg 1987). Even with the use of this technique, many data sets in which the means differed substantially still indicated no significant differences among the means (P=0.05). This

situation is known to occur when there are large differences in the number of plants per treatment, as is the case here.

Crosses with F_1 *V. darrowi* x *V. arboreum* hybrids as the female parent

The fruit set of the F_1 x highbush crosses was 20x greater and significantly different (P=0.04) from the F_1 x V. arboreum and the F_1 x V. darrowi crosses (Table 4). The fruit set for the 7 F_1 x V. arboreum crosses ranged from 0% to 5.1% (Table 5). Only 1 cross out of 4 in the F_1 x V. darrowi crosses produced any fruit, 4.6%. The percent fruit set for the F_1 x highbush crosses ranged from 2.8% to 67.9%.

There was no significant difference (Table 4) in the mean berry weight of the 3 crosses; however the mean berry weight of the cross using highbush pollen was approximately 2x greater than that of the crosses using V. arboreum and V. darrowi pollen. Many factors influence berry weight, including the genetic constitution of the mother plant for berry size, the number of viable ovules present, availability of genetically compatible pollen, and the number of viable seeds. Since all the F_1 clones produced berries with seeds in at least 1 of the crosses (Table 6), it is reasonable to assume that all of these F_1s produced some viable ovules. The most likely reason here for the

difference in berry weight is the number of viable seeds produced by the cross.

Over all, crosses in which the F_1s were the female parent, the mean seed weight per berry was positively correlated with mean berry weight (Table 4). The crosses using V. arboreum pollen had the lowest mean berry weight and the lowest mean seed weight. The crosses with highbush pollen had the highest mean berry weight and the highest mean seed weight. The crosses with V. darrowi pollen were in between the other 2 crosses for mean berry weight and mean seed weight. The mean berry weight for the F_1 x highbush cross was very close to the mean berry weight for the open-pollinated F_1 (Chapter 3, Table 3). The mean number of plump seeds per berry related to mean berry weight in the same manner as mean seed weight per berry.

The number of seedlings per 100 pollinated flowers is the best data by which to judge the success of a cross (Galletta, 1975). This character takes into account the fertility of the parents and the ability of the parental genotypes to work together to produce a viable seedling. The F_1 (V. darrowi x V. arboreum) x highbush crosses produced an average of 3.4 seedlings per 100 pollinated flowers (Table 4). This number was much larger than for the crosses in which the F_1 was crossed with V. arboreum and V. darrowi, 0.2 and 0.4 respectively.

The crosses with highbush pollen produced significantly more plump seeds per 100 pollinated flowers (P=0.01) and more mg seed per 100 pollinated flowers (P=0.02) than the other 2 crosses (Table 4,5,6,and 7). It seems reasonable that the number of plump seeds per 100 pollinated flowers or berries and the mg of seeds per 100 pollinated flowers or berries would give the same estimate of crossing success as the number of seedlings per 100 pollinated flowers. This however was not always true. In this case, the V. arboreum cross produced twice as many plump seeds per 100 pollinated flowers as the V. darrowi cross. The opposite was true for these 2 crosses for seedlings per 100 pollinated flowers and mg seed per 100 pollinated flowers; V. darrowi is twice that of V. arboreum. These data indicated that many plump seeds were inviable and that the number of plump seeds per 100 pollinated flowers was not a good estimate of cross success for these wide hybrids. A prime example of this was the cross 85-131 x 'Avonblue' (a highbush cultivar) which produced 192.7 plump seeds per 100 pollinated flowers but only 14.3 seedlings per 100 pollinated flowers (Table 5).

MIK and MIK derivative crosses

The MIKs were uses as female parents in 2 types of crosses, MIK x MIK and MIK x highbush. One (MIK x MIK) clone was crossed with highbush. Five (MIK x HB) clones were backcrossed to highbush. Ten highbush x highbush

Table 4. Summary of data collected from F_1 (*V. darrowi* x *V. arboreum* hybrids) controlled crosses made in 1992, 1993, and 1994. Numbers are the average of all available data.

Cross	n[z]	Fruit set (%)	Seedlings per 100 pollinated flowers	Berry weight (g)	Seed per berry (mg)	Plump seeds per 100 pollinated flowers	Plump seeds per berry	Weight of 10 large seeds (mg)	Seed per 100 pollinated flowers (mg)
F_1 x Arb[y]	7	1.3 b[y]	0.2	0.13	1.8	1.4 b[x]	1.0 b[x]	---	1.2 b[w]
F_1 x Dar[t]	4	1.2 b	0.4	0.08	0.6	0.7 b	0.2 b	---	0.7 b
F_1 x HB[s]	11	20.8 a	3.4	0.24	2.9	70.4 a	3.3 a	67.1	51.0 a

[z]Number of crosses. The number of flowers pollinated per cross is given in Table 5.
[y]Mean separation within columns by LSMeans with a Tukey-Kramer adjustment on transformed date, P=0.04. Actual means presented here.
[x]P=0.01
[w]P=0.02
[v]*V. arboreum*
[t]*V. darrowi*
[s]Highbush

Table 5. Number of flowers pollinated, fruit set and number of seedlings for F_1 (*V. darrowi* x *V. arboreum* hybrids) controlled crosses made in the spring of 1993 and 1994.

Cross	Type of cross	Flowers pollinated	Fruit set[z] (%)	Seedlings per 100 pollinated flowers
85-131 x 93-1	F_1 x Arb[y]	500	2.8	0.8
85-127 x Boulware #2		500	0.2	0.0
85-134 x 93-1		500	0.0	0.0
85-130 x 93-2		500	0.2	0.4
85-131 x Boulware #2		532	5.1	0.0
85-127 x 93-4 + Boulware #2		475	1.1	0.0
85-134 x 93-2		645	0.0	0.0
85-131 x 91-318	F_1 x Dar[x]	500	4.6	1.4
85-127 x 91-331		500	0.0	0.0
85-134 x 91-317		500	0.0	0.0
85-130 x 91-323		500	0.0	0.0
85-130 x 'Sharpblue'	F_1 x HB[w]	800	2.8	3.9
85-131 x 93-36		568	21.5	1.2
85-131 x 'O'Neal'		256	42.6	8.6
85-131 x 3-8 + 78-15		826	17.2	0.6
85-131 x 'Avonblue'		28	67.9	14.3
85-134 x 93-67		992	7.1	0.4
85-134 x 90-210		1363	9.8	1.0
85-134 x 78-15		870	21.0	2.0
85-134 x 'Avonblue'		1052	10.9	0.3
85-127 x 'Avonblue'		208	17.8	0.5
85-127 x 90-210		261	10.7	4.2

[z]Number of fruit matured divided by number of flowers pollinated.
[y]*V. arboreum*
[x]*V. darrowi*
[w]Highbush

Table 6. Berry weight, seed per berry, and number of plump seeds for F_1 (*V. darrowi* x *V. arboreum* hybrids) controlled crosses made in the spring of 1993 and 1994.

Cross	Type of cross	Berry weight[z] (g)	Seed per berry (mg)	Plump seeds per 100 pollinated flowers	Plump seeds per berry[y]
85-131 x 93-1	F_1 x Arb[x]	0.11[w]	0.6	2.5	0.9[v]
85-127 x Boulware #2		---[u]	2.5	0.0	1.0[t]
85-134 x 93-1		+[s]	+	0.0	0.0
85-130 x 93-2		0.11[r]	4.3	0.6	3.0[r]
85-131 x Boulware #2		0.12[q]	0.9	5.8	1.1[q]
85-127 x 93-4 + Boulware #2		0.18[p]	0.9	1.1	1.0[p]
85-134 x 93-2		+	+	0.0	0.0
85-131 x 91-318	F_1 x Dar[o]	0.08[n]	0.6	2.9	0.6[m]
85-127 x 91-331		+	+	0.0	0.0
85-134 x 91-317		+	+	0.0	0.0
85-130 x 91-323		+	+	0.0	0.0
85-130 x 'Sharpblue'	F_1 x HB[l]	0.10[k]	2.5	5.9	2.1[k]
85-131 x 93-36		0.13	2.0	70.2	3.3
85-131 x 'O'Neal'		0.19	3.0	190.3	4.7
85-131 x 3-8 + 78-15		0.13	7.2	43.8	2.8
85-131 x 'Avonblue'		0.28[j]	2.1	192.7	2.8[j]
85-134 x 93-67		0.27	1.2	13.2	1.9
85-134 x 90-210		0.31	0.2	35.7	3.6
85-134 x 78-15		0.33	1.9	90.4	4.3
85-134 x 'Avonblue'		0.27	1.8	33.1	3.0

Table 6 continued.

Cross	Type of cross	Berry weight[z] (g)	Seed per berry (mg)	Plump seeds per 100 pollinated flowers	Plump seeds per berry[y]
85-127 x 'Avonblue'	F_1 x HB	0.31[y]	4.2	52.8	3.0
85-127 x 90-210		0.35[q]	6.0	46.1	4.3[q]

[z]Average of 50 berries
[y]Average of 30 berries
[x]V. arboreum
[w]Average of 7 berries
[v]Average of 9 berries
[u]---Data not collected
[t]1 berry
[s]+No material to take data on
[r]Average of 10 berries
[q]Average of 27 berries
[p]Average of 5 berries
[o]V. darrowi
[n]Average of 20 berries
[m]Average of 23 berries
[l]Highbush
[k]Average of 22 berries
[j]Average of 19 berries

Table 7. Weight of large seeds, number of seeds per pollinated flower and plump seeds per berry for F_1 (*V. darrowi* x *V. arboreum*) hybrids) controlled crosses made in the spring of 1993 and 1994.

Cross	Type of cross	Weight of 10 large seeds[z] (mg)	Seed per 100 pollinated flowers (mg)	Berries with plump seeds /berries without plump seeds
85-131 x 93-1	F_1 x Arb[x]	*[x]	1.7	3/9
85-127 x Boulware #2		*	0.5	1/0
85-134 x 93-1		+[w]	0.0	0/0
85-130 x 93-2		*	0.9	1/0
85-131 x Boulware #2		*	4.4	18/9
85-127 x 93-4 + Boulware #2		*	1.0	4/1
85-134 x 93-2		+	0.0	0/0
85-131 x 91-318	F_1 x Dar[v]	*	2.7	9/14
85-127 x 91-331		+	0.0	0/0
85-134 x 91-317		+	0.0	0/0
85-130 x 91-323		+	0.0	0/0
85-130 x 'Sharpblue'	F_1 x HB[u]	*	6.8	18/4
85-131 x 93-36		15.2[t]	42.1	105/5
85-131 x 'O'Neal'		17.1[t]	129.0	104/3
85-131 x 3-8 + 78-15		13.6[s]	17.6	142/0
85-131 x 'Avonblue'		18.2[r]	142.5	19/0
85-134 x 93-67		507.2[t]	8.2	62/11
85-134 x 90-210		20.1[s]	18.3	117/15
85-134 x 78-15		20.9[s]	39.6	157/16
85-134 x 'Avonblue'		18.9[t]	20.2	109/5

Table 7 continued.

Cross	Type of cross	Weight of 10 large seeds[z] (mg)	Seed per 100 pollinated flower (mg)	Berries with plump seeds /berries without plump seeds
85-127 x 'Avonblue'	F_1 x HB[u]	21.1[q]	0.7	32/3
85-127 x 90-210		18.6[s]	0.6	38/2

[z]Average of 5 replications
[y]V. arboreum
[x]*Not enough material for 1 replication.
[w]+No material to take data on
[v]V. darrowi
[u]Highbush
[t]Average of 2 replications.
[s]Average of 3 replications.
[r]1 replication.
[q]Average of 4 replications.

crosses were made to have a standard of comparison for the MIK and MIK derivative crosses.

The mean percent fruit set of the MIK x MIK and MIK x HB crosses (Table 8) were inflated by the occurrence in 1992 of 7 crosses that produced more fruit than the number of flowers pollinated (Table 9). If these clones were counted as having a fruit set of 100%, the mean percent fruit set for the MIK x MIK crosses would be 66.7% and 70.3% for the MIK x HB crosses. Fruit set greater than 100% did not occur for any clone in subsequent years and is discussed in Chapter 5.

Using the adjusted mean percent fruit sets, there was a small but steady increase in fruit set with increasing

amounts of highbush genes in the clones. However, the (MIK x HB) x HB clones at 69.0%, were slightly below that of the (MIK x MIK) x HB clones at 70.3%. This may have been due to sampling error, since only 5 (MIK x HB) x HB crosses were made. Three of the 5 clones in this group had percent fruit sets within the range of the HB x HB crosses (Table 9). The ranges of fruit set for the MIK and MIK derivative crosses was quite large. All had plants with very high percent fruit sets and plants with very low percent fruit sets. By comparison, the highbush x highbush percent fruit sets did not have any extreme lows and were all clustered between 65% and 100%.

The mean berry weight also increased as more highbush genes were incorporated into the hybrid plants. There was no significant difference (P=0.01) between the MIK x MIK, MIK x HB and (MIK x MIK) x HB crosses in mean berry weight. There was, however, a trend toward increasing mean berry weight from 0.71g to 0.88g to 1.00g. The (MIK x HB) x HB crosses were significantly different (P=0.01) from the other MIK crosses, but not from the HB x HB crosses. Although not statistically different from the HB x HB mean berry weight, the (MIK x HB) x HB mean berry weight was still too low for commercial standards. Individual plants however, had berries large enough to meet cultivar standards. The data indicate that good progress is being made toward increasing

Table 8. Summary of data collected from controlled crosses made in 1992, 1993, and 1994. Numbers are averages of all available data.

Cross	n[z]	Fruit set (%)	Seedlings per 100 pollinated flowers	Berry weight (g)	Seed per berry (mg)	Plump seeds per 100 pollinated flowers	Plump seeds per berry	Weight of 10 large seeds (mg)	Seed per 100 pollinated flowers (mg)
MIK[y] x MIK	10	77.2	83.7	0.71b[x]	12.8	312.4[w]	6.1[w]	10.6[w]	1170.9
MIK x HB[v]	15	73.1	85.6	0.88b	14.1	1081.1[u]	13.3[u]	11.7[u]	1153.6
(MIK x MIK) x HB	1	93.0	68.0	1.00ab	12.2	995.1	10.7	8.1	1136.2
(MIK x HB) x HB	5	69.0	44.0	1.49a	4.9	719.0	9.8	8.9	352.6
HB x HB	10	81.4	230.5	1.73a	13.1	1991.4	23.8	6.2	1081.9

[z]Number of crosses. The number of flowers pollinated per cross is given in Table 9.
[y]Open-pollinated progeny of F_1 hybrids
[x]Mean separation within columns by LSMeans with a Tukey-Kramer adjustment, P=0.01.
[w]2 replications
[v]Highbush
[u]6 replications

Table 9. Number of flowers pollinated, fruit set, and number of seedlings for controlled crosses made in the spring of 1992, 1993 and 1994.

Cross	Type of cross	Flowers pollinated	Fruit set[z] (%)	Seedlings per 100 pollinated flowers
91-400 x 89-104	MIK[y] x MIK	298	70.8	100.0
89-100 x 89-106		300	107.0	77.0
89-104 x 89-108		300	110.7	184.0
89-105 x 91-333		301	50.8	30.0
89-106 x 91-333		300	27.3	27.0
89-107 x 89-100		300	110.7	152.0
89-108 x 91-400		300	79.0	0.0
91-333 x 89-107		300	113.3	218.0
93-128 x 93-90		183	48.1	22.5
93-90 x 178		200	54.0	26.4
89-105 x 83-135	MIK x HB[x]	308	69.8	30.0
89-106 x 'Marimba'		311	21.5	23.0
89-107 x NC 1528		300	99.3	174.0
89-108 x 'Avonblue'		300	110.0	0.0
91-333 x 'O'Neal'		300	126.7	177.0
91-400 x NC 1528		112	51.8	91.0
89-100 x 6-19		300	78.7	104.0
89-104 x NC 1523		303	104.6	268.0
91-401 x 6-19		135	6.4	11.0
93-136 x 90-4		200	62.5	67.2
93-128 x 90-4		200	67.0	82.7
89-100 x 90-178		200	62.0	48.9
93-90 x 'Avonblue'		200	80.0	25.1
91-333 x 3-8		200	83.5	170.0
93-135 x 'Rebel'		200	72.0	12.5

Table 9 continued.

Cross	Type of cross	Flowers pollinated	Fruit set[z] (%)	Seedlings per 100 pollinated flowers
93-89 x 'Rebel'	(MIK x MIK) x HB	200	93.0	268.0
93-132 x 83-135	(MIK x HB) x HB	200	29.0	21.5
93-129 x 'Marimba'		200	63.0	7.5
93-131 x 3-8		200	78.5	39.8
93-134 x 90-178		200	79.0	113.3
93-130 x 83-135		200	95.5	37.8
93-56 x 3-8	HB x HB	100	70.0	98.6
91-198 x 90-103		101	89.1	184.5
93-41 x 87-217		194	80.9	153.4
93-76 x 93-81		87	79.3	136.9
93-73 x 85-58		83	79.5	376.0
93-71 x 83-135		100	81.0	406.8
93-42 x TH275		74	67.6	39.2
93-68 x 6-19		76	85.5	120.7
5-12 x 93-29		100	82.0	161.3
93-74 x 85-58		78	98.7	627.2

[z]Number of fruit matured divided by number of flowers pollinated.
[y]Open-pollinated progeny of F_1 hybrids
[x]Highbush

Table 10. Berry weight, seed per berry, and number of plump seeds for controlled crosses made in the spring of 1992, 1993, and 1994.

Cross	Type of cross	Berry weight[z] (g)	Seed per berry (mg)	Plump seeds per 100 pollinated flowers	Plump seeds per berry[y]
91-400 x 89-104	MIK[x] x MIK	0.98	17.9	---[w]	---
89-100 x 89-106		0.66	13.5	---	---
89-104 x 89-108		0.58	16.7	---	---
89-105 x 91-333		0.47	4.9	---	---
89-106 x 91-333		0.47	6.8	---	---
89-107 x 89-100		1.01	24.6	---	---
89-108 x 91-400		0.56	1.1	---	---
91-333 x 89-107		0.80	28.5	---	---
93-128 x 93-90		0.82	6.7	290.0	6.0
93-90 x 178		0.79	7.3	334.8	6.2
89-105 x 83-135	MIK x HB[v]	0.58	4.7	---	---
89-106 x 'Marimba'		0.46	7.5	---	---
89-107 x NC 1528		0.93	22.3	---	---
89-108 x 'Avonblue'		0.63	1.8	---	---
91-333 x 'O'Neal'		0.83	26.6	---	---
91-400 x NC 1528		1.11	19.7	---	---
89-100 x 6-19		0.66	14.7	---	---
89-104 x NC 1523		0.66	25.8	---	---
91-401 x 6-19		0.91	5.0	---	---
93-136 x 90-4		1.15	13.3	1373.4	21.8
93-128 x 90-4		1.01	10.9	891.1	13.3
89-100 x 90-178		1.02	10.3	652.9	10.5
93-90 x 'Avonblue'		1.00	15.7	992.0	2.4
91-333 x 3-8		1.38	31.9	2419.0	29.0
93-135 x 'Rebel'		0.86	1.3	158.4	2.2

Table 10 continued.

Cross	Type of cross	Berry weight[z] (g)	Seed per berry (mg)	Plump seeds per 100 pollinated flowers	Plump seeds per berry[y]
93-89 x 'Rebel'	(MIK x MIK) x HB	1.00	12.2	995.1	10.7
93-132 x 83-135	(MIK x HB) x HB	0.64	4.1	268.8	9.3
93-129 x 'Marimba'		1.59	2.3	201.6	3.2
93-131 x 3-8		1.47	4.5	779.5	9.9
93-134 x 90-178		1.94	7.2	1027.0	13.0
93-130 x 83-135		1.83	6.6	1317.9	13.8
93-56 x 3-8	HB x HB	1.15	15.1	935.9	13.4
91-198 x 90-103		1.64	11.1	1957.7	22.0
93-41 x 87-217		2.01	10.5	1052.1	13.0
93-76 x 93-81		1.46	16.7	2342.0	29.5
93-73 x 85-58		1.72	16.9	1890.1	23.8
93-71 x 83-135		1.84	18.4	5316.0	65.6
93-42 x TH275		1.30	4.0	522.3	7.7
93-68 x 6-19		2.00	8.3	715.9	8.4
5-12 x 93-29		2.40	8.5	989.7	12.1
93-74 x 85-58		1.75	21.3	4192.6	42.5

[z]Average of 50 berries
[y]Average of 30 berries
[x]Open-pollinated progeny of F_1 hybrids
[w]Data not collected
[v]Highbush

Table 11. Weight of large seeds, mg of seeds per 100 pollinated flowers and plump seeds per berry for controlled crosses made in the spring of 1992, 1993, and 1994.

Cross	Type of cross	Weight of 10 large seeds[z] (mg)	Seed per 100 pollinated flowers (mg)	Berries with plump seeds /berries without plump seeds
91-400 x 89-104	MIK[y] x MIK	---[x]	1266.9	---
89-100 x 89-106		---	1452.3	---
89-104 x 89-108		---	1853.7	---
89-105 x 91-333		---	247.5	---
89-106 x 91-333		---	187.8	---
89-107 x 89-100		---	2720.1	---
89-108 x 91-400		---	27.6	---
91-333 x 89-107		---	3234.9	---
93-128 x 93-90		11.0	321.9	85/0
93-90 x 178		10.2	396.5	107/1
89-105 x 83-135	MIK x HB[w]	---	328.1	---
89-106 x 'Marimba'		---	162.7	---
89-107 x NC 1528		---	2211.4	---
89-108 x 'Avonblue'		---	65.9	---
91-333 x 'O'Neal'		---	3370.7	---
91-400 x NC 1528		---	1019.3	---
89-100 x 6-19		---	1157.7	---
89-104 x NC 1528		---	2700.0	---
91-401 x 6-19		---	78.3	---
93-136 x 90-4		12.0	833.9	125/0
93-128 x 90-4		10.8	729.7	131/0
89-100 x 90-178		11.9	637.8	118/0
93-90 x 'Avonblue'		13.0	1256.8	156/0
91-333 x 3-8		13.3	2660.5	167/0
93-135 x 'Rebel'		8.9[v]	90.8	75/67

Table 11 continued.

Cross	Type of cross	Weight of 10 large seeds[z] (mg)	Seed per 100 pollinated flowers (mg)	Berries with plump seeds /berries without plump seeds
93-89 x Rebel	(MIK x MIK) x HB	8.1	1136.2	197/0
93-132 x 83-135	(MIK x HB) x HB	8.7	119.9	57/1
93-129 x 'Marimba'		10.9[u]	126.0	117/10
93-131 x 3-8		7.9	350.9	137/18
93-134 x 90-178		10.0	567.7	130/30
93-130 x 83-135		7.1	598.5	148/41
93-56 x 3-8	HB x HB	7.1	1056.3	30/0
91-198 x 90-103		5.9	989.9	30/0
93-41 x 87-217		6.6	853.8	29/0
93-76 x 93-81		6.7	1327.2	30/0
93-73 x 85-58		5.7	1345.5	30/0
93-71 x 83-135		4.8	1488.0	30/0
93-42 x TH275		6.9	272.7	30/0
93-68 x 6-19		6.5	709.9	30/0
5-12 x 93-29		5.6	687.0	30/0
93-74 x 85-58		6.2	2089.1	30/0

[z]Average of 5 replications
[y]Open-pollinated progeny of F_1 hybrids
[x]Data not collected
[w]Highbush
[v]1 replication.
[u]Average of 4 replications

the berry size of MIK derivatives in only a few generations of backcrossing.

Plump seeds per 100 pollinated flowers, seed weight per berry, number of plump seeds per berry and mg seed per 100 pollinated flowers are all estimates of female fertility. There was no significant difference (P=0.05) between the MIK x MIK, MIK x HB, (MIK x MIK) x HB and HB x HB crosses for mean mg seed per berry and mean mg seed per 100 pollinated flowers (Table 8). The (MIK x HB) x HB means for these 2 characters was less than half that of the other taxa. As stated before, this may be due to the low number of replications for this type of cross. In general, plants that produce more seed by weight are considered to be more fertile than similar plants that produce less seed. Seed weight in this study was confounded by the much larger seed size of the hybrid taxa as compared to southern highbush (Chapter 3, Table 3). This made mg of seed per berry and mg of seed per 100 pollinated flowers not very useful in estimating female fertility.

There were no significant differences (P=0.05) between any of the crosses for mean number of plump seeds per berry and mean number of plump seeds per 100 pollinated flowers (Table 8 and 10). However, there was a general trend toward increasing seediness for both characters as more highbush genes were added to the hybrid taxa through backcrossing.

There were approximately twice as many plump seeds per berry produced by the MIK x HB crosses as the MIK x MIK crosses. The HB x HB crosses produced approximately twice as many plump seeds per berry as the MIK x HB crosses. The number of plump seeds per 100 pollinated flowers followed the same trend.

The number of seedlings produced is the best measure of the success of a cross. This character takes into account female fertility, male fertility, and how well the parental genes combine and work together to form a vigorous seedling. The MIK x MIK and MIK x HB crosses produced similar numbers of seedlings, averaging 83.7 and 85.6 seedlings per 100 pollinated flowers respectively (Table 8 and 9). The single (MIK x MIK) x HB cross produced 3x this amount at 268 seedlings per 100 pollinated flowers. The (MIK x HB) x HB crosses produced a average of 44.0 seedlings per 100 pollinated flowers (Table 8). This was half that of the MIK x MIK and MIK x HB crosses.

Cytogenetics

Chromosomes were counted for 3 F_1s: 85-127, 85-134, and 85-130. A minimum of 2 separate counts of 24 chromosomes were made for each clone (Table 12), indicating that these clones were diploid as expected.

Table 12. Chromosome counts for plants representing the F_1 (*V. darrowi* x *V. arboreum* hybrids) and MIK (open-pollinated progeny of F_1 hybrids) taxa.

Plant	Taxa	Chromosome Number
85-127	F_1	24
85-134		24
85-130		24
93-135	MIK	<48
93-128		48
91-333		48
93-136		48

Chromosome counts were made for 5 MIKs that were chosen for their high fertility. Forty-eight chromosomes were counted for each of 3 MIKs: 93-128, 91-333, and 93-136. These MIKs were tetraploid as was suggested by a comparison of their morphology to the F_1 parents. Seven counts were made for clone 93-135. Chromosome counts for this clone ranged from 42-47. The variable counts may have been due to no perfect cells being found to count or it is possible that the clone may have been a mixaploid. Aneuploidy may explain some of the low fertility in the MIKs. It was not possible to get a good chromosome spread for 93-90, and no count was possible.

Table 13. Mean total seed weight from 50 berries of the 5 taxa used in this study. Fifty berries were harvested from each of 20 plants in each taxon.

Taxa	Number of plants	Total seed weight from 50 berries (mg)
V. darrowi	20	498 ab[z]
V. arboreum	20	366 b
Highbush	20	878 a
F_1[y]	5	67 c
MIK[x]	20	783 a

[z]Means within columns separated by LSMeans with a Tukey-Kramer adjustment on transformed data, P=0.01. Actual means are presented here.
[y]V. darrowi x V. arboreum hybrids
[x]Open-pollinated progeny of F_1 hybrids

Total Seed Weight

The total seed weight of 50 berries was measured for V. darrowi, V. arboreum, southern highbush, F_1s, and the MIKs (Table 13). The total seed weight of 50 berries was measured for each of 20 plants for each taxa. Only 5 of the F_1 clones produced enough berries for this study. Although seed weight per berry is sometimes used as an indicator of female fertility, it was not a good indicator in this study. This was due to the fact that individual seeds were much larger for V. arboreum than for highbush and V. darrowi (Chapter 3, Table 3). Southern highbush and V. darrowi typically have many small seeds, whereas V. arboreum has

fewer seeds that are much larger. The F_1s, MIKs and MIK derivatives inherited the large seed size of V. arboreum. Total seed weight of 50 berries would be a good index of fertility only if there was no difference in seed size.

The mean total seed weight of 50 berries for the F_1s, 0.067 mg, was significantly different (P=0.01) from the V. arboreum parent, 0.366 mg (Table 13). Even though individual seeds were much larger in the F_1 hybrids than in the V. darrowi parent, total seed weight per 50 berries was much higher for V. darrowi due to low seed numbers in the F_1s. The total seed weight was much higher for MIKs, 0.783 mg, than for their F_1 parents, 0.067 mg. The difference in seed size of the MIKs and southern highbush makes a comparison of total seed weight difficult to interpret. Despite the confounding factor of seed size and the limitations it imposes, these data show a drastic decrease in female fertility of the F_1 hybrids over the V. arboreum parent. They also demonstrated a return to much higher fertility levels in the next generation, the MIKs.

Pollen

Pollen stainability is a technique used to investigate male fertility. As expected, the F_1s had the lowest mean pollen stainability at 0.9% (Table 14). In addition to low stainability, most of the pollen grains were shrunken

Table 14. Pollen stainability and amount of pollen.

Taxa	Number of plants	Pollen stainability (%)	Amount of pollen
Highbush	20	87.6 a[z]	4.2
V. arboreum	20	78.3 a	2.7
V. darrowi	20	84.9 a	4.2
(MIK[y] x HB[x])	111	75.1 a	3.2
(MIK selfed)	16	48.8 b	2.4
(MIK x MIK)	56	45.8 b	2.5
MIK	64	43.7 b	2.5
F_1[w]	15	0.9 c	1.7

[z]Means within columns separated by LSMeans with a Tukey-Kramer adjustment on transformed data, P=0.01. Actual means are presented here.
[y]Open-pollinated progeny of F_1 hybrids
[x]Highbush
[w]V. darrowi x V. arboreum hybrids

(Figure 17). The MIKs and (MIK x MIK) clones did not differ significantly (P=0.01). These 2 taxa showed a large increase in mean pollen stainability (Table 14 and Figure 18) over the F_1s. All of the hybrid taxa, except the F_1s, contained individuals that had percent pollen stainabilities in the nineties. There was no significant difference (P=0.01) between V. arboreum, V. darrowi, southern highbush and the (MIK x HB) taxa. This indicates a return to normal male fertility levels for the MIK backcrosses to southern highbush in only 2 generations.

The amount of pollen produced by a plant is also a measure of male fertility. The amount of pollen was rated

Figure 17. Shrunken, non-staining pollen grains characteristic of F_1 (*V. darrowi* x *V. arboreum*) hybrids.

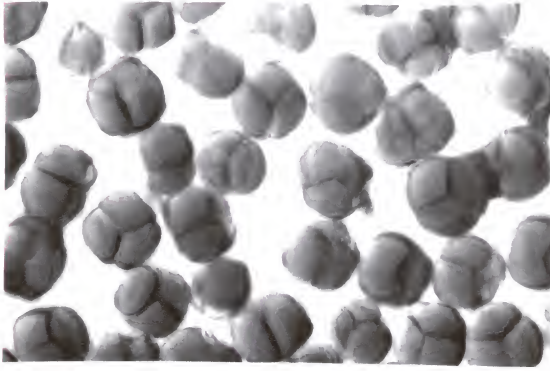

Figure 18. Plump, stained pollen grains found in the fertile MIKs (open-pollinated progeny of the F_1 hybrids).

on a scale of 0-5, with 0 indicating no pollen and 5 indicating a copious pollen flow. The F_1s had the lowest mean amount of pollen at 1.7 (Table 14). All of the F_1 clones produced at least a small amount of pollen. The amounts ranged from 1-3, with 1 being a very low amount of pollen and 3 being a medium amount of pollen. Not only was the average amount of pollen produced very low, the majority of pollen grains were inviable as indicated by nonstaining. The mean amount of pollen for the MIKs and (MIK x MIK) taxa was greater than the F_1s and less than the (MIK x HB) taxa. Highbush and V. darrowi had the highest mean amount of pollen. Surprisingly, V. arboreum had a lower mean amount of pollen than the other non-hybrid taxa, and it was close to that of the MIKs and (MIK x MIK) taxa.

Summary

Many of the MIKs were sterile or had very low fertility levels (also discussed in Chapter 6, Tables 23-26). One possible reason for the low fertility is aneuploidy. The chromosome counts of MIK clone 93-135 showed that aneuploidy was probably present in the MIK population. Another possible explanation for low fertility could be ploidy level. During the bloom time of the F_1 hybrids, possible pollen sources included diploids, tetraploids, and

hexaploids. These MIKs could potentially be haploid, triploid or pentaploid.

The controlled crosses with the F_1 hybrids pointed to southern highbush as the pollen parent of the original MIKs. The F_1 crosses with southern highbush were much more successful than the crosses with V. arboreum and V. darrowi for every character measured. Mean percent fruit set was 20x greater for the highbush crosses than for the other 2 types of crosses. The F_1 x highbush crosses produced 8.5x more seedlings than the F_1 x V. darrowi crosses and 17x more seedlings than the F_1 x V. arboreum crosses.

Male and female fertility of the MIK derivatives increased with increasing amounts of southern highbush in the parentage. This was more evidence that highbush was the pollen parent of the original MIKs. Mean percent fruit set, mean number of seedlings per 100 pollinated flowers, mean berry weight, mean pollen stainability and mean amount of pollen all increased through this series of crosses. None of the populations as a whole met the criteria set by the southern highbush crosses in any of the fertility indicators measured. Yet, every taxon had individual plants that were within the ranges established by the highbush x highbush crosses as normal fertility levels. The 1 exception was mean pollen stainability for the (MIK x HB) taxa, which was

not statistically different (P=0.01) from the highbush x highbush crosses.

It was shown here that mg seed per berry, plump seeds per 100 pollinated flowers, number of plump seeds per berry, and mg of seed per 100 pollinated flowers were not good estimates of female fertility. The characters that involve weight measurements of seeds were confounded by the difference in seed size between the MIKs, MIK derivatives and highbush. The number of plump seeds per 100 pollinated flowers might be expected to have a direct correlation with number of seedlings per 100 pollinated flowers. This, however, did not appear to be true for any of the taxa, even highbush. Only about an eighth of the plump highbush seeds produced seedlings. It should be noted that a 12.5% germination rate for highbush was lower than what has been reported in the literature. A germination rate of 35% (Lyrene, 1988) is closer to normal. This may indicate that not all viable seeds germinated in the test year. However, since all seeds were subjected to the same environment, comparisons should still be valid.

There was a dramatic increase in fertility from the F_1s to the MIKs. The increase in female fertility was shown in the large increase in total seed weight for 50 berries, percent fruit set, berry weight, and number of seedlings per 100 pollinated flowers. There was also a dramatic increase

in mg of seed per berry, number of plump seeds per 100 pollinated flowers, number of seeds per berry and mg of seed per 100 pollinated flowers. Since there was no significant difference (Chapter 3, Table 3) between seed size for the F_1s and MIKs, characteristics involving seed weight were not confounded by seed size. Thus, fertility comparisons involving seed weight were valid in this instance.

Male fertility also increased dramatically from the F_1s to their MIK progeny as evidenced by pollen stainability and amount of pollen produced.

The dramatic increase in fertility along with increased flower and leaf size was a good indicator that the MIKs are tetraploid, rather than diploid like their F_1 parent. This hypothesis was supported by the chromosome counts made on several MIKs.

CHAPTER 5
SELF-FERTILITY AND PARTHENOCARPY

Introduction

One purpose of making controlled crosses in the greenhouse was to identify any possible anomalies that could appear in the progeny of a wide cross, e.g. self-compatibility. Seven of the 23 MIK crosses produced in 1991 had fruit sets greater than 100% of the number of flowers hand pollinated, the excess fruit coming from flowers that were not hand pollinated but were not removed from the plant. Since most blueberry clones produce little or no fruit from unpollinated flowers under the conditions that prevailed in this experiment, the excess fruit set sparked an interest in the possibility of increased self-compatibility or parthenocarpy in these clones. One result of wide hybridization can be the production of abnormal F_1 plants due to the breakdown of complex gene systems in normal plants. Examples include albino plants, dwarfs, and male and/or female sterility. It was hypothesized that the high fruit set in these hybrid clones might indicate a breakdown in the blueberry self-incompatibility system.

The possibility of self compatibility or parthenocarpy was investigated by counting the number of plump seeds in each of the first 30 berries harvested from each cross and determining how many of the other berries contained no plump seeds. Additional hand self-pollinations were made to see if the exceptional fruit sets could be repeated. Fruit set was examined on 2 MIKs that were placed on a bench in a bee-proof greenhouse and not hand pollinated. Several clones of southern highbush, V. darrowi and V. arboreum were selfed to establish standards for comparison purposes.

Materials and Methods

Plants to be used in greenhouse crosses were dug from the Horticultural Unit at the University of Florida, Gainesville, Florida, in December 1991, 1992, and 1993. The plants were potted in peat and placed in a cooler kept at approximately 5C. Plants remained in the cooler for a minimum of 1 month to satisfy their chilling requirements. This promoted synchronous blooming, making crossing of plants with different chilling requirements possible. From the cooler, plants were moved to a bee-proof greenhouse where the temperature was maintained between 5C and 27C.

Flowers were pollinated on or near the day of anthesis. On the day of pollination, the corolla and anthers were removed. Pollen was applied to the stigma from the

thumbnail. The number of flowers pollinated varied for each cross. Before pollination, the male parent was checked for pollen stainability to ensure that low fruit set would not be due to inviable pollen.

In the spring of 1994, 1 plant each of the MIK clones 89-107 and 91-333 were placed on a bench in a bee-proof greenhouse without hand-pollination to estimate self-fruitfulness. Flowers on each plant were counted and ripe fruit were harvested.

Ripe berries were harvested daily and stored in a cold room if necessary. Data were collected on fruit set and number of plump seeds. The first 30 berries harvested from each cross were kept separate from the remaining harvest. These berries were squashed individually on paper towels. Seeds were dried overnight on a laboratory bench. The following day the number of plump seeds per berry were counted and recorded. As the remaining berries were harvested, they were treated in the manner just described. However, instead of counting all the plump seeds, a record was made of the presence or absence of plump seeds in each berry.

Results and Discussion

The MIK clones with fruit sets greater than 100% in 1992 were: 91-333, 89-104, 89-108, 89-100, and 89-107

(Table 15). Three of the crosses that gave more than 100% fruit set were MIK x HB and 4 were MIK x MIK. All of the crosses with these clones produced percent fruit sets and number of seedlings per 100 pollinated flowers greater than the overall cross means for MIK x MIK, MIK x HB, and MIK x self crosses (Table 15). The mean berry weight for the crosses varied with each cross; some were greater than and some were less than the mean berry weights for that type of cross.

Vaccinium darrowi and V. arboreum were low in all measures of self-fertility (Table 16). Only 1 V. darrowi x self cross produced any fruit (Table 17). The mean berry weight after this self-pollination was 0.18 g. This was very close to the mean berry weight, 0.20 g, of open-pollinated V. darrowi plants in the field (Chapter 3, Table 3). Only 1 of the 3 V. arboreum x self crosses produced fruit. As with V. darrowi, the mean berry weight for the V. arboreum self was very close to the mean berry weight for open-pollinated V. arboreum in the field, 0.27 g for the berries produced by selfing and 0.28 g for berries in the field.

More than half of the berries produced by the V. arboreum x self cross lacked plump seeds (Table 19). In contrast, the V. darrowi x self crosses produced 41 berries, all of which contained plump seeds. The mean number of

seedlings per 100 pollinated flowers, mg of seed per berry, plump seeds per 100 pollinated flowers, plump seeds per berry, and mg seed per 100 pollinated flowers were all much lower than for any of the other crosses (Table 16, 17, 18, and 19 and Chapter 4, Tables 4 and 8).

The highbush x self crosses had a mean fruit set of 76.7% (Table 16). This was close to the mean fruit set for highbush x highbush crosses at 81.4% (Chapter 4 Table 8). The mean berry weight of the highbush selfs was 1.22 g. This wqs less than the highbush x highbush mean berry weight of 1.73 g, but still within the lower end of the range. Only 1 cross produced any berries without plump seeds, and this was only 1 berry. For all the other parameters measured, the highbush selfs were much lower, at least 50%, than the highbush x highbush crosses.

The MIK x self crosses had a mean fruit set of 37.2% (Table 15). This is approximately half the mean fruit set of the MIK x MIK crosses. The mean berry weight of the self crosses was 0.67 g, only slightly less than the MIK x MIK mean berry weight of 0.71 g. For all the other parameters measured, the MIK selfs were much lower than the MIK x MIK crosses. Only 1 cross, 93-128 x self, produced a large proportion of berries without plump seeds, 60% (Table 19).

The (MIK x HB) x self crosses had a mean percent fruit set approximately 40% less than the mean percent fruit set

Table 15. Summary of data from MIK (open-pollinated progeny of F_1 hybrids) crosses that produced greater than 100% fruit set in 1992. All plants were hand pollinated.

MIK[z]	Fruit set (%)			Seedlings per 100 pollinated flowers			Berry weight (g)		
	HB[yx]	MIK[y]	Self[y]	HB[y]	MIK[y]	Self[y]	HB[y]	MIK[y]	Self[y]
91-333	126.7	113.3	99.0	177.0	218.0	67.0	1.38	0.82	0.86
89-104	104.6	110.7	--	268.0	184.0	--	0.66	0.58	--
89-108	110.0	79.0	86.0	0.0	0.0	0.0	0.63	0.56	0.52
89-100	78.7	107.0	--	104.0	77.0	--	0.67	0.66	--
89-107	99.3	110.7	84.3	174.0	174.0	150.0	0.93	0.60	0.90
Mean of all MIK crosses[w]	73.1	77.2	37.2	85.6	83.7	19.5	0.88	0.71	0.67

[z]Female parent
[y]Male parent
[x]Highbush
[w]Data taken from Table 16 and Chapter 4 Table 8.

Table 16. Summary of data collected from controlled crosses made in 1992, 1993, and 1994. Numbers presented are the average of all available data.

Cross	n[z]	Fruit set (%)	Seedlings per 100 pollinated flowers	Berry weight (g)	Seed per berry (mg)	Plump seeds per 100 pollinated flowers	Plump seeds per berry	Weight of 10 large seeds (mg)	Seed per 100 pollinated flowers (mg)
Arb[y] selfed	3	7.8	0.0	0.27 c[x]	1.2	1.8 b	0.03	---[w]	3.9 b
Dar[v] selfed	3	7.0	2.5	0.18 c	1.3	28.5 ab	1.37	---	9.4 ab
MIK[u] selfed	20	37.2	19.5	0.67 bc	7.5	168.2 ab	4.66	12.3	312.6 ab
(MIK x HB[t]) selfed	5	41.7	11.4	0.92 abc	2.9	214.3 ab	3.54	11.4	182.6 ab
HB selfed	3	76.7	70.5	1.22 a	7.1	602.0 a	8.80	---	492.1 a

[z]Number of crosses. The number of flowers pollinated per cross is given in Table 17.
[y]*V. arboreum*
[x]Mean separation within columns by LSMeans with a Tukey-Kramer adjustment on transformed data, except for berry weight, P=0.05. Actual means are presented here.
[w]Data not taken.
[v]*V. darrowi*
[u]Open-pollinated progeny of F_1 hybrids
[t]Highbush

Table 17. Number of flowers pollinated, fruit set and number of seedlings for controlled crosses made in the spring of 1992, 1993, and 1994.

Cross	Type of cross	Flowers pollinated	Fruit set (%)	Seedlings per 100 pollinated flowers
Boulware Arb #2	Arb[z] selfed	200	10.5	0.0
Boulware Arb #4		209	0.0	0.0
93-1		256	12.9	0.0
91-313	Dar[y] selfed	200	0.0	0.0
91-323		201	0.0	0.0
91-331		200	21.0	7.5
89-100	MIK[x] selfed	200	82.0	9.0
89-107		200	37.5	9.2
91-333		200	75.0	10.0
93-128		195	49.7	7.0
93-135		190	19.5	1.1
93-136		200	25.0	3.5
93-137		77	29.9	0.0
93-90		200	60.5	22.4
94-21		18	0.0	0.0
94-14		4	0.0	0.0
94-18		30	0.0	0.0
94-19		139	13.9	5.8
94-16		200	1.0	2.5
94-17		200	13.5	7.5
89-105		300	7.0	3.0
89-106		301	16.6	23.0

Table 17 continued.

Cross	Type of cross	Flowers pollinated	Fruit set (%)	Seedlings per 100 pollinated flowers
89-107	MIK selfed	300	84.3	150.0
89-108		300	86.0	0.0
91-333		300	99.0	67.0
91-400		298	44.3	60.0
93-133	(MIK x HB[w]) selfed	100	57.0	7.8
93-134		200	85.0	40.3
93-131		200	45.5	6.5
93-130		182	19.8	1.6
93-132		200	1.0	1.0
5-12	HB selfed	100	94.0	11.0
93-71		100	58.0	62.4
93-56		100	78.0	138.2

[v]V. arboreum
[y]V. darrowi
[x]Open-pollinated progeny of F_1 hybrids
[w]Highbush

Table 18. Berry weight, seed per berry, and number of plump seeds for controlled crosses made in the spring of 1992, 1993, and 1994.

Cross	Type of cross	Berry weight (g)[z]	Seed per berry (mg)	Plump seeds per 100 pollinated flowers	Plump seeds per berry[y]
Boulware Arb #2	Arb[x] selfed	0.27[w]	1.1	5.5	0.1[v]
Boulware Arb #4		+[u]	+	0.0	0.0
93-1		+	+	0.0	0.0
91-313	Dar[t] selfed	+	+	0.0	0.0
91-323		+	+	0.0	0.0
91-331		0.18[s]	1.3	85.5	4.1
89-100	MIK[r] selfed	1.04	3.7	162.8	4.0
89-107		0.98	5.7	332.6	8.9
91-333		0.86	6.7	600.0	8.0
93-128		0.78	6.1	301.9	6.1
93-135		0.45[y]	0.3	25.9	1.3
93-136		0.51[q]	3.8	215.8	8.6
93-137		0.47[w]	2.1	134.4	4.5[p]
93-90		0.84	10.1	514.3	8.5
94-21		+	+	0.0	0.0
94-14		+	+	0.0	0.0
94-18		+	+	0.0	0.0
94-19		0.15[o]	0.9	11.2	1.6[n]
94-16		0.98[m]	6.5	10.5	10.5[m]
94-17		0.59[w]	16.7	45.0	3.3[l]
89-105		0.34	3.6	---[k]	---

Table 18 continued.

Cross	Type of cross	Berry weight (g)[z]	Seed per berry (mg)	Plump seeds per 100 pollinated flowers	Plump seeds per berry[y]
89-106	MIK selfed	0.45	6.4	---	---
89-107	(MIK x HB[j]) selfed	0.90	17.0	---	---
89-108		0.52	0.9	---	---
91-333		0.74	20.3	---	---
91-400		0.78	14.7	---	---
93-133		1.16	5.9	340.3	6.0
93-134		1.53	6.2	606.1	7.1
93-131		0.77	1.0	109.2	2.4
93-130		0.85[y]	0.3	14.4	0.7
93-132		0.27[m]	1.1	1.5	1.5[m]
5-12	HB selfed	1.42	1.4	373.2	4.0
93-71		1.23	10.1	889.1	15.3
93-56		1.00	9.7	543.7	7.0

[z]Average of 50 berries
[y]Average of 30 berries
[x]V. arboreum
[w]Average of 20 berries
[v]Average of 21 berries
[u]+No material to take data on
[t]V. darrowi
[s]Average of 25 berries
[r]Open-pollinated progeny of F_1 hybrids
[q]Average of 40 berries
[p]Average of 22 berries
[o]Average of 5 berries
[n]Average of 9 berries
[m]Average of 2 berries
[l]Average of 27 berries
[k]---Indicates data not taken
[j]Highbush

Table 19. Weight of large seeds, mg seeds per pollinated flowers, and plump seeds per berry for controlled crosses made in the spring of 1992, 1993, and 1994.

Cross	Type of cross	Weight of 10 large seeds[z] (mg)	Seed per 100 pollinated flowers (mg)	Berries with plump seeds/berries without plump seeds
Boulware Arb #2	Arb[y] selfed	*[x]	11.8	9/12
Boulware Arb #4		+[w]	0.0	0/0
93-1		+	0.0	0/0
91-313	Dar[v] selfed	+	0.0	0/0
91-323		+	0.0	0/0
91-331		---[u]	28.2	41/0
89-100	MIK[t] selfed	12.2	149.6	80/3
89-107		---	231.7	75/0
91-333		15.2	499.6	150/0
93-128		11.5[s]	302.1	97/0
93-135		---	6.5	16/24
93-136		10.5	95.5	45/6
93-137		---	61.4	16/6
93-90		12.9	611.4	123/1
94-21		+	0.0	0/0
94-14		+	0.0	0/0
94-18		+	0.0	0/0
94-19		*	6.5	8/2
94-16		*	6.5	2/0
94-17		11.4[r]	22.6	25/2
89-105		---	25.3	---

Table 19 continued.

Cross	Type of cross	Weight of 10 large seeds (mg)	Seed per 100 pollinated flowers (mg)	Berries with plump seeds/berries without plump seeds
89-106	MIK selfed	---	107.1	---
89-107		---	1430.0	---
89-108		---	25.5	---
91-333		---	2010.3	---
91-400		---	650.1	---
93-133	(MIK x HB[q]) Selfed	16.5	333.7	56/0
93-134		9.5	526.0	159/11
93-131		8.3[s]	46.6	81/11
93-130		*	6.4	16/18
93-132		*	1.1	2/0
5-12	HB selfed	---	135.4	29/1
93-71		---	585.8	30/0
93-56		---	755.1	30/0

[z]Average of 5 replications
[y]*V. arboreum*
[x]*Indicates not enough material for a full replication
[w]+No material to take data on
[v]*V. darrowi*
[u]---Indicates data not taken
[t]Open-pollinated progeny of F_1 hybrids
[s]Average of 3 replications
[r]1 replication
[q]Highbush

for the highbush x self crosses and approximately 45% less than the highbush x highbush crosses (Table 16 and Chapter 4, Table 8). The number of seedlings per 100 pollinated flowers was considerably less than the highbush x self crosses, 11.4 vs 70.5. The mean berry weight of the (MIK x HB) selfs was not significantly different from the highbush selfs. The remaining measures of self fertility may not be valid comparisons. Seed size was not measured for the (MIK x HB) taxa in the morphology study. However, since the MIKs had a significantly larger seed size than highbush, it seemed best not to make the comparisons.

Six plants that were the progeny of MIK x self crosses were themselves selfed. Three of the plants had a parent that gave over 100% fruit set in 1992; 94-14 came from 91-333 x self, 94-16 and 94-17 came from 89-107 x self. The other 3 plants were from MIK x self crosses that were not exceptional in percent fruit set; 94-21 came from 89-106 x self, 94-18 and 94-19 came from 91-400 x self.

The percent fruit set of all 6 crosses was exceptionally low (Table 17). Three of the selfs produced no fruit. These plants produced a very low number of flowers so this may have contributed to the lack of fruit set. The other 3 clones produced a large number of flowers but had very low fruit sets; 1%, 13.5% and 13.9%. The number of seedlings produced per 100 pollinated flowers was

also quite low; 5.8, 2.5 and 7.5. Because all 6 plants were the result of selfing, another contributing factor to low fruit set and seedling production could have been inbreeding depression.

Two MIK clones, 91-333 and 89-100, were crossed with highbush in 1993. The MIK x HB cross 91-333 x 3-8 cross had a fruit set of 83.5% (Chapter 4, Table 9). The previous year, the 91-333 x MIK cross gave a fruit set of 126.7% and the 91-333 x highbush cross had a fruit set of 113.3% (Table 15). For all 3 crosses the number of seedlings per 100 pollinated flowers was at least double that of the mean for MIK x MIK crosses (Table 15).

MIK clone 89-100 x 89-106 (MIK x MIK) gave a fruit set of 107.0% in 1992. The following year 89-100 x 90-178 (MIK x HB) had a fruit set of 62.0%. The number of seedlings per 100 pollinated flowers was 77 the first year and 48.9 the second year. The mean berry weight of the MIK x MIK cross was 0.66 g and the mean berry weight of the MIK x HB cross was 1.02 g. Both of these weights are in keeping with the combined mean berry weight of the crosses in these 2 categories.

MIK clones 89-107 and 91-333 were chosen to be left unpollinated in the greenhouse in 1994. These clones were selected because they both had fruit sets of over 100% in 1992.

After this treatment, 89-107 had a fruit set of 4.2% and 91-333 had a fruit set of 12.8% (Table 20). This was quite a bit lower than the MIK x self crosses which had a mean fruit set of 37.2% (Table 15). The number of seedlings per 100 non-pollinated flowers was 3.8 for 89-107. 91-333 produced 34.6 seedlings per 100 non-pollinated flowers. This was 1.7x larger than the MIK x self mean seedling number of 19.5, but less than half the seedling number of the MIK x MIK crosses. Neither clone produced a significant number of parthenocarpic berries (Table 20).

The mean berry weight of 89-107, which was left in the greenhouse without hand-pollination, was less than the overall mean for MIK x self crosses (Table 16). Yet it had approximately twice as many plump seeds per berry as the MIK x self crosses. 91-333, also left in the greenhouse without hand-pollination, had a mean berry weight of 1.06 g. This was greater than the overall mean for the MIK x self crosses and the MIK x MIK crosses. The mean number of plump seeds per berry from 91-333 not hand-pollinated was 41.5, much larger than the overall mean number of plump seeds per berry for the MIK x MIK crosses of 6.1. It is twice the overall mean number of plump seeds per berry for the highbush x highbush crosses at 23.8 (Chapter 4, Table 8).

Table 20. Data collected from MIK (open-pollinated progeny of F_1 hybrids) clones that were not hand pollinated in the greenhouse the spring of 1994.

Female parent	Number of flowers on plant	Fruit set (%)	Seedlings per 100 flowers	Berry weight[z] (g)	Seed per berry (mg)	Plump seeds per 100 flowers	Plump seeds per berry[y]	Weight of 10 large seeds[x] (mg)	Seed per 100 flowers (mg)	Berries with plump seeds /berries without plump seeds
89-107	1325	4.2	3.8	0.40	3.4	34.2	8.1	15.1[w]	18.4	53/3
91-333	1450	12.8	34.6	1.06	8.9	532.3	41.5	15.5	164.6	172/14

[z]Average of 50 berries
[y]Average of 30 berries
[x]Average of 5 replications
[w]Average of 4 replications

Summary

Fruit set from blueberry flowers that were not cross-pollinated has 2 possible origins: self-pollination, which would produce seeded fruit, or parthenocarpy, which would yield seedless-fruit. Fruit set as a result of unassisted self-pollination in blueberry would be favored by 2 conditions. The first of these is the deposition on the stigma of substantial quantities of self pollen. This would be favored by copious pollen flow combined with a favorable flower structure. The flower structure would favor self pollination if the anther and stigmas were closer together than normal for that taxon. MIK clones 91-333 and 89-107 were checked in 1994 and did not exhibit unusually close anthers and stigmas. The second condition that would favor fruit set due to self-pollination would be an increased level of self-compatibility.

The results of this study indicated that the exceptional fruit set, greater than 100%, of the 5 MIK clones in 1992 was probably due to unassisted self-pollination of some flowers on these plants. Why this occurred at such a high rate in these clones in 1992 is not known. Fruit sets this high were not repeated in subsequent years by these clones or any other clones. There was no indication that the MIKs or MIK derivatives are any more

self-compatible than highbush. There was also no indication of a greater tendency for parthenocarpic berries.

CHAPTER 6
OPEN-POLLINATED FIELD STUDY

Introduction

The crosses that produced this nursery were made in the spring of 1990. The progeny of these crosses were used primarily to gather field data on open-pollinated MIKs and MIK derivatives. The plants were located in a high density nursery, surrounded by tetraploid highbush.

Materials and Methods

The plants (Table 21) to be used in these greenhouse crosses were dug from the Horticultural Unit at the University of Florida, Gainesville on December 20, 1989. MIKs were selected to be parents based on having had high fruit set in seedling nurseries in the field the previous spring. The plants were potted in peat and placed in a cooler at approximately 5C. Plants remained there for a minimum of 1 month to satisfy their chilling requirements. At the end of this time, plants were placed in a bee-proof greenhouse where the temperature was maintained between 5C and 27C. On the day of pollination, the corolla and anthers

were removed. Pollen was applied to the stigma from the thumbnail. Ripe berries were harvested and stored in a cold room if necessary. After harvest was complete, the seeds were removed using a food blender. Seeds were dried overnight on a paper towel in the laboratory. Berries from 4 V. darrowi x V. arboreum F_1s, open-pollinated in a field containing a large collection of rabbiteye and highbush selections, (Table 21) were harvested and handled in the same manner as berries from the greenhouse crosses.

Table 21. Seedling populations evaluated in a high density field nursery in 1992.

Type of cross	Parents	Taxa of progeny	Plants per cross
F_1[z] OP[y]	85-130[x]	MIK[w]	16(11)[v]
F_1 OP	85-131[x]	MIK	38(23)
F_1 OP	85-134[x]	MIK	13(2)
F_1 OP	85-127	MIK	21(2)
MIK selfed	89-107 selfed	MIK selfed	55(20)
MIK x MIK	89-107 x 89-100	MIK x MIK	87(54)
MIK x HB[u]	89-105 x 3-8	MIK x HB	8(4)
MIK x HB	89-106[x] x 86-14	MIK x HB	11(2)
MIK x HB	89-100 x 'Georgia Gem'	MIK x HB	21(15)
MIK x HB	89-107 x 'Avonblue'	MIK x HB	61(55)
MIK x HB	89-104 x 85-93	MIK x HB	27(13)
MIK x HB	89-108 x 86-8	MIK x HB	65(43)

[z]V. darrowi x V. arboreum hybrid
[y]Open-pollinated
[x]'Johnblue', a V. darrowi cultivar, is in the pedigree.
[w]Open-pollinated progeny of the F_1s
[v]Number of plants that flowered
[u]Highbush

Seeds were planted in November, 1990. The night before planting, seeds were soaked in 10.4 mM gibberellic acid for approximately 12 hours (Dweikat and Lyrene, 1989a). Seeds were sown on peat, without covering, in pots with a 10 cm top diameter. The pots were placed in a greenhouse where the temperature was maintained between 5C and 27C. A mist system was used for watering. In February, 1991, the seedlings were transplanted to flats. These were maintained in a greenhouse until early May, when the seedlings were transplanted into a high density nursery at the Horticultural Unit at the University of Florida, Gainesville, Florida.

In 1992, these plants were allowed to open-pollinate in the field. Data were collected on flower number, fruit set, pollen stainability, berry weight, bloom date, ripening date, and seed weight. In 1993, data were again taken on flower number. Due to several late freezes (Feb. 18 and 19, March 13 and 14, 1993), no additional data were taken.

In 1992, the number of flowers and amount of fruit were rated as high, medium, low or zero for every bush. Flowers for studying pollen stainability were collected, stored and examined in the manner previously described. Five berries per plant were weighed. Seeds were removed from berries as previously described.

Results and Discussion

The average bloom date (Table 22) for the new MIKs (seedlings from open-pollinated seed harvested from *V. darrowi* x *V. arboreum* F_1s) was late March in 1992. The F_1s normally bloom from mid February through April. Their extremely long bloom time is characteristic. The MIK average bloom date was later than southern highbush which normally blooms from late January through early March in Gainesville, Florida (Lyrene and Sherman, 1978; Lyrene and Sherman, 1992), but earlier than *V. arboreum* and *V. darrowi*, which bloom late March through early April. The average bloom date for the (MIK x HB) seedlings was mid-March, which was intermediate between the parental taxa.

The average harvest date of the new MIKs (F_1 OP in Table 22) was intermediate between the F_1s and the hypothesized southern highbush parent. Mean ripening date for the MIKs was late May, whereas the F_1s ripened in June and July and highbush ripened April through mid-May (Lyrene and Sherman, 1978; Lyrene, 1989). The late ripening date of the F_1s can be attributed to the influence of genes from *V. arboreum*, since *V. arboreum* ripens in October. The MIK derivative taxa (MIK x MIK and MIK x HB) ripened from mid-May through mid-June.

Table 22. Data taken on seedling populations in a high density field nursery in the spring of 1992. The berry data were from berries produced after open-pollination. All data are the population means.

Pedigree and type of cross	Average bloom date	Average harvest date	Mean berry weight (g)	Berry weight range (g)	Pollen stainability (%)
85-130 OP[z] (F_1[y] OP)[x]	3/25	5/18	0.75	0.42-0.99	40.7
85-131 OP (F_1 OP)	3/30	5/31	0.58	0.15-0.94	45.4
85-134 OP (F_1 OP)	3/28	5/25	0.86	0.86	40.4
85-127 OP (F_1 OP)	3/21	5/22	0.88	0.88	43.8
F_1 OP mean			0.77		42.6
89-107 selfed (MIK[w] selfed)	3/26	5/27	0.84	0.52-1.31	48.8
89-107 x 89-100 (MIK x MIK)	3/18	5/27	0.89	0.48-1.69	46.6
89-105 x 3-8 (MIK x HB)	---[v]	6/9	0.85	0.50-1.43	---
89-106 x 86-14 (MIK x HB[u])	---	6/8	0.56	0.55-0.56	---
89-100 x 'Georgia Gem' (MIK x HB)	3/17	5/18	1.10	0.55-1.71	82.1
89-107 x 'Avonblue' (MIK x HB)	3/11	5/22	1.10	0.59-2.04	80.3
89-104 x 85-93 (MIK x HB)	3/9	5/18	0.96	0.49-1.81	65.2
89-108 x 86-8 (MIK x HB)	3/14	5/22	1.25	0.61-2.02	70.7
(MIK x HB) mean			0.97		74.6

[z]Open-pollinated
[y]*V. darrowi* x *V. arboreum* hybrid
[x]Progeny from this source are called "MIKs"
[w]Open-pollinated progeny of F_1 hybrids
[v]Data not collected
[u]Highbush

The overall mean berry weight (0.77 g) for the 4 MIK populations was similar to that of the MIK x MIK and MIK x HB controlled crosses in the greenhouse (Chapter 4, Table 8). The mean berry weights of these 4 MIK populations after open-pollination ranged from 0.58 g to 0.88 g.

The overall mean berry weight of the 6 MIK x HB populations was 0.97 g. Mean berry weights for individual crosses ranged from 0.49 g to 2.04 g. A wide range in weights should be expected due to the diverse genotypes a cross of this nature is likely to produce, and due to the fact that many clones would be expected to be only partially fertile. The mean berry weight of the HB x HB controlled crosses was 1.72 g (Chapter 4, Table 8). Four of the 6 populations had some plants that produced berries close to or exceeding this mean. This matched the berry weight of 'Sharpblue' (Lyrene 1989), an industry standard.

MIK clone 89-107 was used as the female parent in 3 different types of crosses. It was selfed, crossed with another MIK, and crossed with the highbush cultivar 'Avonblue'. The crosses became increasingly more fertile with additional amounts of highbush genes being introduced into the progeny genome.

The mean berry weight, in the open-pollinated nursery, of the (89-107 x self) and (89-107 x MIK) populations were similar, 0.84 g and 0.89 g, respectively. However the range

of individual plants in the (89-107 x MIK) population was 0.48-1.69 g, whereas it was 0.52-1.31 g for the (89-107 x self) population. The greatest mean berry weight for an individual plant of the (89-107 x MIK) populations was 1.69 g. This was essentially identical to the mean berry weight of the open-pollinated highbush clones, 1.65 g, examined in the morphology study (Chapter 3, Table 3). The mean berry weight of the (89-107 x HB) population was 1.10 g, a 0.21 g increase over the (89-107 x MIK) population. The berry weight of the plants at the upper end of the range from the (89-107 x HB) population matched the mean berry weight for 'Sharpblue'.

The mean pollen stainability (Table 22) of the progeny populations also increased through this series of crosses. As for mean berry weight, the (89-107 x self) and (89-107 x MIK) populations were similar in mean pollen stainability, 48.8% and 46.6%, respectively. However, the 2 populations differed in their ranges. The (89-107 x self) population contained plants that ranged from 0% to 75.0%. The (89-107 x MIK) progeny ranged from 0% to 97.5% stainability. The mean pollen stainability of the (89-107 x HB) plants was 80.3%, almost double that of the other 2 crosses. The range of pollen stainability among plants in the (89-107 x HB) population extended slightly beyond that of the (89-107 x MIK) population, 0% to 99.0%. Each of these crosses

produced individual plants with pollen stainabilities that were within the range of fertile non-hybrid taxa.

Flower number, berry number and pollen stainability were plotted (Tables 23-32) for each cross in an effort to determine if male and female fertility were correlated. Flower number in conjunction with berry number was used in this study as a measure of female fertility. It must be kept in mind that the production of berries requires not only female fertility, but also fertilization by genetically compatible pollen. The presence of flowers does not necessarily indicate viable ovules; however the absence of berries on plants that flowered in the presence of plentiful viable pollen does indicate a lack of female fertility. Pollen stainability was used here as a measure of male fertility.

The tables are read by examining each box. Each datum within each box is percent pollen stainability for 1 plant. The row in which each percentage occurs indicates the estimated quantity of flowers on that plant. The column each percentage occupies indicates the estimated amount of berries on the same plant. Thus, the lower box on the right side of Table 23 indicates that there was 1 plant with 27.1% pollen stainability and that this plant had a low number of flowers and produced no berries.

Tables 23-32 give little evidence that male and female fertility were correlated in the progeny of any of the crosses undertaken. It was possible to find individuals that ranged from a high berry set and 0% pollen stainability (Table 26) to individuals with no berry set and 86.6% pollen stainability (Table 30). The tables showed wide variability within each progeny population for male and female fertility. There was however, a trend toward higher female and male fertility as increasing amounts of the southern highbush genome are incorporated into the hybrids.

Summary

The average harvest date became earlier as the generations advanced from F_1 to MIK to MIK derivatives. The F_1s were extremely late ripening due to the genetic influence of their V. arboreum parent. Their low seediness may also have delayed their maturity. With increasing amounts of southern highbush in the pedigree, the harvest date became earlier. The same was true of average bloom date, but the shift was not as great.

Mean berry weight for the populations increased with increasing percentage of southern highbush in the parentage. The MIK derivative populations contained some individuals that met cultivar standards for berry weight.

MIK clone 89-107 was selfed, crossed with another MIK, and crossed with highbush. The progeny populations showed an increase in berry weight and pollen stainability with increasing amounts of highbush in their pedigree. These data provide further evidence that highbush is the pollen parent of the MIKs produced by open-pollination.

Table 23. Male and female fertility data for the open-pollinated progeny of F_1 clone 85-130. Berries were the result of open-pollination in the field. Each percentage figure represents 1 plant.

Flower number	Berry number			
	High	Medium	Low	0
High	66.0%[z] 59.0%	75.0% 0.0%	92.1% 55.0%	21.9% 12.0%
Medium			18.5%	22.7% 5.5%
Low			73.3%	27.1%

[z]Percent pollen stainability

Table 24. Male and female fertility data for the open-pollinated progeny of F_1 clone 85-131. Berries were the result of open-pollination in the field. Each percentage figure represents 1 plant.

Flower number	Berry number			
	High	Medium	Low	0
High		76.0%[z] 41.5%	73.4%	44.3% 0.0%
Medium		87.5% 70.4%	83.3% 40.1%	29.0% 0.0% 0.0%
Low			77.5%	73.5% 57.2% 17.5% 0.0%

[z]Percent pollen stainability

Table 25. Male and female fertility data for the open-pollinated progeny of F_1 clone 85-134. Berries were the result of open-pollination in the field. Each percentage figure represents 1 plant.

Flower number	Berry number			
	High	Medium	Low	0
High	73.8%[z]			
Medium				7.0%
Low				

[z]Percent pollen stainability

Table 26. Male and female fertility data for the open-pollinated progeny of F_1 clone 85-127. Berries were the result of open-pollination in the field. Each percentage figure represents 1 plant.

Flower number	Berry number			
	High	Medium	Low	0
High				
Medium			79.5%[z]	
Low				8.0%

[z]Percent pollen stainability

Table 27. Male and female fertility data for the progeny of cross 89-107 (MIK) selfed. Berries were the result of open-pollination in the field. Each percentage figure represents 1 plant.

Flower number	Berry number			
	High	Medium	Low	0
High		72.0%[z] 52.2% 49.5%	75.0%	63.7% 46.5% 32.5%
Medium		0.0%	69.5% 57.0% 55.0% 0.0%	69.3% 40.1%
Low			50.0%	48.0%

[z]Percent pollen stainability

Table 28. Male and female fertility data for the open-pollinated progeny of 89-107 x 89-100 (MIK x MIK). Berries were the result of open-pollination in the field. Each percentage figure represents 1 plant.

Flower number	Berry number			
	High	Medium	Low	0
High	97.5%[z] 82.2% 82.0% 80.1% 78.6% 75.6% 71.1% 63.5% 56.7% 56.5% 34.8% 27.9% 18.3% 16.3% 6.0% 0.0% 0.0%	85.6% 79.7% 61.9% 60.0% 49.0% 47.0% 36.0% 32.5% 25.2% 23.3% 14.3% 1.5% 0.0% 0.0% 0.0%	73.0% 41.4% 32.8% 1.0% 0.0%	
Medium		87.0% 75.5%	95.0% 84.7% 78.1% 77.1% 68.0% 61.2% 49.5% 39.6% 34.8% 0.0%	68.0% 4.0% 1.5%
Low			84.5%	25.7%

[z]Percent pollen stainability

Table 29. Male and female fertility data for the open-pollinated progeny of the cross 89-100 x 'Georgia Gem' (MIK x HB). Berries were the result of open-pollination in the field. Each percentage figure represents 1 plant.

Flower number	Berry number			
	High	Medium	Low	0
High	98.0%[z] 85.6% 74.6% 68.8%	94.5% 91.1% 80.6% 67.8%	91.1%	
Medium		86.0% 84.2%	79.5%	
Low				65.0%

[z]Percent pollen stainability

Table 30. Male and female fertility data for the open-pollinated progeny of the cross 89-104 x 89-93 (MIK x HB). Berries were the result of open-pollination in the field. Each percentage figure represents 1 plant.

Flower number	Berry number			
	High	Medium	Low	0
High		59.6%[z]	91.6% 86.5%	
Medium		76.1%		38.8%
Low			85.1%	71.6% 47.3%

[z]Percent pollen stainability.

Table 31. Male and female fertility data for the open-pollinated progeny of 89-107 x 'Avonblue' (MIK x HB). Berries were the result of open-pollination in the field. Each percentage figure represents 1 plant.

Flower number	Berry number			
	High	Medium	Low	0
High	95.5%[z] 93.5% 90.5% 87.6% 87.2% 86.5% 86.1% 86.0% 85.6% 85.6% 85.0% 84.1% 82.6% 79.5% 78.7% 78.2% 74.1% 73.9% 73.3% 72.3% 69.2% 67.8% 65.5% 0.0%	99.0% 89.6% 88.1% 87.6% 83.6% 75.0% 74.3% 72.5% 52.7%	96.5% 91.0% 90.0% 79.5% 78.6% 77.6% 75.5%	
Medium		97.5% 95.5% 94.0% 70.0% 39.5%	93.0% 77.2% 72.3% 43.3%	
Low			76.8% 72.0%	69.7%

[z]Percent pollen stainability

Table 32. Male and female fertility for the open-pollinated progeny of the cross 89-100 x 86-8 (MIK x HB). Berries are the result of open-pollination in the field. Each percentage figure represents 1 plant.

Flower Number	Berry Number			
	High	Medium	Low	0
High	98.0%[z] 93.1% 93.0% 90.6% 90.5% 88.2% 87.5% 86.1% 75.2% 74.0% 65.5% 60.0% 54.5% 50.7% 0.0% 0.0%	92.6% 86.0% 83.7% 78.5% 72.8% 68.0% 60.6% 53.7%	80.3% 79.6%	
Medium		94.0% 84.7% 68.0%	88.1% 79.6% 74.9% 74.1% 63.5% 41.8%	
Low			91.5% 43.6% 43.3% 27.9%	86.6%

[z]Percent pollen stainability

CHAPTER 7
ISOZYMES

Introduction

One of the primary uses of isozyme analysis is to show whether or not suspected hybrids are actually hybrids. This method is more objective than searching for intermediate morphology. Isozyme markers show a co-dominant inheritance, whereas dominance in genes affecting morphological traits could cause F_1 hybrids to resemble their parents. The objective of this study was to demonstrate in an objective manner that the F_1 hybrids were produced by a cross between V. arboreum and V. darrowi.

Materials and Methods

Most of the V. darrowi clones used as female parents were no longer available. However, the V. darrowi cultivar 'Johnblue' was the female parent of 6 of the 16 V. darrowi x V. arboreum F_1 hybrids. Therefore, isozyme analysis was run on 4 of these 6. The specific clones of V. arboreum that were used as male parents were also not available because pollen for the crosses was obtained by cutting flowering

branches from trees in the wild. The trees were located in Alachua and Santa Rosa Counties, Florida, but notes on the specific location of these trees were not made. Because of this, it was decided to sample the local Alachua County V. arboreum population. Twenty different genotypes were selected from a total of 5 different locations: San Felasco Hammock Reserve in Gainesville, Horticultural Unit at the University of Florida in Gainesville, O'Leno State Park near High Springs, Paynes Prairie State Park in Micanopy and potted plants in the blueberry breeding program greenhouse. These were then considered to be representative of the local genotypes.

Leaf tissue was used for analysis. Leaves were collected from the field or greenhouse and placed on ice until they could be transported to a laboratory refrigerator.

The extraction buffer used consisted of 100 mM Tris-HCl (pH 7.5), 7% sucrose (w/v), 10% PVP-40 (w/v), 14 mM mercaptoethanol (0.1% v/v), 250 mM ascorbic acid Na-salt, 20 mM diethyldithiocarbamate, 1.0% bovine serum albumin (w/v), 20 mM sodium metabisulfite, and 200 mM sodium tetraborate (Wendel and Weeden, 1989). The buffer was prepared fresh each time samples were prepared, and was refrigerated until use. Cold leaf tissue was ground in a pre-chilled mortar and pestle with the buffer (5:1, w/v). Wicks made from #3

Whatman filter paper were dipped in the crude extract, wiped quickly on a Kim-Wipe and inserted into a microfuge tube. The tubes were placed in an ice bath until the extractions had been completed, and then transferred to a -80 C freezer for storage.

Three enzyme systems were explored, but only 1 gave good resolution and revealed banding differences useful for this study. Malate dehydrogenase (MDH E.C. 1.1.1.37) could not be resolved. 6-Phosphogluconate dehydrogenase (PGD, E.C. 1.1.1.44) resolved well, but did not show a difference between the parents. Glucose-6-phosphate isomerase (Pgi, E.C. 5.3.1.9) was resolved and showed clear differences between the V. arboreum and V. darrowi parents.

Gels were prepared using 8.3% potato starch, 4% sucrose and 10 mg NAD. The gel and electrode buffer system used was lithium-borate/tris-citrate, both at a pH of 8.3 (Soltis et al., 1983). The electrode buffer consisted of 0.188 M boric acid and 0.038 M lithium hydroxide. The gel buffer was composed of 0.045 M Tris-HCl, 0.007 M citric acid and 100 ml of the electrode buffer to make 1 L of buffer solution. The sample wicks were removed from cold storage just prior to loading. Sample insertion into the gel was done as quickly as possible, keeping the gel on an ice pack during the process. Four layers of Handy Wipes were used as the buffer wicks. Gels were electrophoresed in the refrigerator at 5C,

with an ice pack on top of the gel, at a constant amperage of 50 mA for approximately 8 hours using an EC-400 power source. Afterwards, the gels were sliced and stained for Pgi (Soltis et al., 1983) The stain consisted of: 5 ml 1.0 M Tris-HCl pH 8.0, 45 ml distilled de-ionized water, 0.02 g fructose-6-phosphate disodium salt, 20 units glucose-6-phosphate dehydrogenase (NAD), 0.01 g NAD, 0.01 g MTT, and 0.002 g PMS. Staining was complete after 4 hours in the dark at room temperature. After staining, the gels were rinsed in de-ionized water and fixed. The fixative consisted of 1 glycerol:2 acetic acid:4 water:5 ethanol. Fixing took approximately 15 minutes. The gels were then wrapped in plastic wrap and stored in the refrigerator.

Results and Discussion

The survey of V. arboreum showed 2 zones of banding in the plants examined (Figures 19 and 20). Pgi-1 was represented by a single band for all genotypes. Six different zymotypes (Lanes 6-14) were observed in the Pgi-2 zone. The banding patterns of the 6 zymotypes indicated the presence of 4 different alleles, designated a, b, c, and d, at the Pgi-2 locus. Three of the possible homozygous zymotypes, (aa, bb, and dd) were not found in the populations evaluated. The Pgi-2 genotypes resolved were: a'c, ac, ad, bc, cc, and cd (see below). The 3-banded

pattern of the heterozygotes was consistent with the dimeric structure of Pgi-2.

One V. arboreum plant (Lane 6) showed 2 Pgi-2 bands, instead of the expected 1 or 3 bands. One of the bands was located at the position indicating the c allele and the other was located at the position indicating the b allele. A similar situation was reported by Vorsa et al. (1988) for 2 V. darrowi clones. They hypothesized an a' allele that was migrating with the Pgi-1 allele, and thus masked. Later van Heemstra et al. (1991) confirmed that there was a Pgi-2 allele that co-migrated with the Pgi-1 allele. The band that co-migrated in this study with the Pgi-1 allele represented the heterodimeric form of the enzyme in the plant in question.

'Johnblue' (Lane 1), the V. darrowi parent of the putative F_1s hybrids examined, exhibited single bands at the Pgi-1 and Pgi-2 loci. The genotype inferred for the Pgi-2 locus was bb. The 4 F_1s (Lanes 2-5) examined exhibited zymotypes that were consistent with their hypothesized hybrid nature. All 4 had a band indicating the a allele that could have been inherited from the V. darrowi parent, 'Johnblue'. The other alleles present in the F_1s were c and d. The zymotypes of the 4 F_1s were: bc, bc, bc and bd.

Figure 19. Isozyme Pgi band patterns of 'Johnblue' (V. darrowi cultivar), F_1 (V. darrowi x V. arboreum hybrids), and V. arboreum. From the left, lane 1 is 'Johnblue', lanes 2-5 are the F_1 hybrids and lanes 6-14 are V. arboreum.

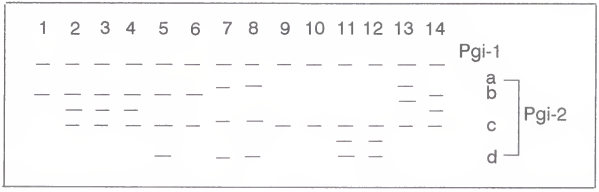

Figure 20. Diagrammatic representation of banding pattern of Pgi isozyme loci. From the left, lane 1 is 'Johnblue' (V. darrowi cultivar), lanes 2-5 are the F_1 (V. darrowi x V. arboreum hybrids), and lanes 6-14 are V. arboreum.

Summary

Isozyme analysis of 4 F_1s and their hypothesized parents, 'Johnblue' and V. arboreum was done. The results of the analysis were consistent with the F_1 hybrids being the progeny of 'Johnblue' and V. arboreum.

CHAPTER 8
PROPAGATION OF *VACCINIUM ARBOREUM*

Introduction

Vaccinium arboreum has been difficult to propagate by seed or cuttings (Stockton, 1976). Several experiments were undertaken to advance knowledge of propagation techniques. One experiment investigated the effectiveness of using gibberellic acid to increase seed germination and another experiment tested the success rate of propagation by softwood cuttings taken from juvenile seedling stock plants. The final experiment investigated survival of seedlings in the greenhouse.

Materials and Methods

The first experiment investigated the effect of using gibberellic acid to promote seed germination. Seeds remaining from the morphology study (Chapter 3) were used in this experiment. Ten of the 20 clones from which open-pollinated seed had been collected from High Springs, Florida, were randomly selected. The seeds from each clone were divided into 2 lots of approximately equal weight. One

lot was treated with a 10 mM solution of gibberellic acid. A small amount of alcohol was added in preparing the solution to promote the dissolution of the gibberellic acid. The other seed lot was soaked in the same solution but without the gibberellic acid.

The night before planting, seeds were immersed in the solutions. The following morning, December 3, 1993, seeds were planted as had previously been described (Chapter 4). Seedlings were counted the following March.

In the second experiment, seedlings were used to produce softwood cuttings. Seeds were germinated in the greenhouse and grown until they were 2 to 5 cm tall and the first true leaves had appeared. On June 8, 1992, the above-ground part of each seedling was cut in half and the upper half was planted in a flat containing peat. The bottom half was discarded. One hundred thirty-two cuttings were taken and divided between 2 flats. The flats were kept in the greenhouse under intermittent mist and shade cloth. The misting schedule was typical of that used in commercial blueberry propagation for softwood cuttings. On July 27, 1992, the flats were removed from the mist beds, but were left under the shade cloth. The seedlings were uprooted on September 16, 1992, and examined for root formation.

The final experiment measured survival of seedlings in the greenhouse environment. Seeds were germinated in the

greenhouse. Once the seedlings were 2-5 cm tall they were transferred into pots (10 cm top diameter) filled with peat. Seedlings were separated into 4 groups based on the origin of the seeds. Four seedlings were planted in each of about 90 pots on June 16, 1992. On August 11, the number of surviving seedlings was counted.

Results and Discussion

The results of the gibberellic acid experiment were the opposite of what was expected. Gibberellic acid is often used to increase the germination rate of hard to germinate seeds. There was no significant difference (P=0.05, paired t-test) in germination between seeds treated with gibberellic acid and seeds not treated. However, in 9 out of 10 seedlots, fewer seedlings were obtained from the gibberellic acid treatment (Table 33). Under the conditions of this experiment, gibberellic acid did not facilitate the germination of V. arboreum seeds. The average seed weight of V. arboreum is approximately 1.35 mg per seed (Chapter 3, Table 3). Using this seed weight as a guide, there were approximately 173 seeds per pot. This information confirms that seed germination was very low in this experiment for both the treated and the untreated seed.

The production of roots was quite high in the seedling softwood cutting experiment. The percentages of cuttings

that produced roots were 91% and 83% for the 2 flats, respectively (Table 34). This experiment indicated that softwood cuttings from juvenile seedlings provide a good method to propagate V. arboreum. These data are in accord with results from other species of difficult-to-root plants, in which juvenile stem cuttings have been found to root much better than those taken from older plants (Hartmann and Kester, 1975).

The survival of seedlings in the greenhouse was also quite good. The percent of seedlings that survived for at least 2 months after germinating ranged from 82-100% (Table 35).

Summary

The data from these experiments indicate that using gibberellic acid to facilitate germination of V. arboreum seeds did not increase seedling production. While there was no statistical difference in germination, 9 out of 10 seedlots not treated with gibberellic acid produced more seedlings that those that were treated.

The seedling softwood cutting experiment showed that juvenile cuttings from 4 month old seedlings of V. arboreum readily form adventitious roots. Once seeds germinated and produced seedlings, survival in the greenhouse was not a problem.

Table 33. Number of V. arboreum seedlings produced by seeds treated with gibberellic acid and not treated.

V. arboreum[z]	Weight of seeds planted (mg)	Number of seedlings
1	202.8	14
1-T[y]	214.7	1
5	250.1	3
5-T	246.5	2
6	247.9	4
6-T	243.6	2
8	170.5	16
8-T	172.4	10
9	243.2	3
9-T	238.5	1
10	315.0	20
10-T	305.8	7
11	199.0	3
11-T	191.1	9
13	332.4	3
13-T	329.4	1
16	195.0	39
16-T	197.4	38
17	184.7	4
17-T	189.2	2

[z]Each number refers to 1 plant from High Springs, Florida, whose open-pollinated seeds were used in the experiment.
[y]T indicates seeds soaked in 10.4 mM gibberellic acid overnight before planting.

Table 34. Number of rooted plants produced by V. arboreum seedling softwood cuttings.

Flat number	Number of cuttings	Percent of rooted cuttings
1	68	91
2	64	83

Table 35. Number of V. arboreum seedlings surviving in the greenhouse.

Seed source (Florida locality)	Number of seedlings potted	Percent of plants surviving
Chattahoochee	11	82
Ponce de Leon	66	82
Micanopy	144	95
Gainesville	136	100

CHAPTER 9
CONCLUSIONS

This research substantiated the hypothesis that the F_1 hybrids are the progeny of crosses between V. darrowi and V. arboreum. Confirmation came from the results of the morphology study, controlled crosses, field data, and isozyme analysis.

There is little doubt that tetraploid highbush was the pollen parent of the MIKs. Evidence for this came from the morphology studies, controlled crosses, field data, and chromosome counts. Increasing male and female fertility with increasing amounts of highbush genes in the MIK derivatives also supported the hypothesis.

Genes from V. arboreum were inherited and expressed in the F_1 and subsequent generations. This was shown by the presence of anther awns, increased seed size, marginal glands and bracteole shape in the hybrid populations.

Knowledge of V. arboreum propagation was furthered. It has been demonstrated that gibberellic acid does not enhance germination of seeds. Though there was not a significant difference ($P=0.05$), fewer seedlings were obtained with the use of gibberellic acid in 9 out of 10 seedlots. Softwood

cuttings of juvenile seedlings readily formed adventitious roots. Survival of seedlings under greenhouse conditions was excellent.

Interest in incorporating V. arboreum genes into cultivated highbush was due to 2 desirable characteristics of sparkleberry: a wide soil tolerance and an open corolla. This study did not investigate soil tolerance of sparkleberry introgressed hybrids. However, Lyrene (1991), reported that MIKs and MIK derivatives thrive on certain soils where highbush grows poorly. Unfortunately, the corolla aperture of the MIKs was not substantially different from that of highbush, although selection in hybrid populations for a wider corolla aperture could be effective.

MIKs and MIK derivatives are currently being used in the University of Florida blueberry breeding program. The best MIK derivatives have so far retained the vigor of their hybrid parents. The recovery of berry size and quality has been remarkably fast. For the best selections in the BC_2 to highbush [(MIK x HB) x HB], berry quality is close to cultivar standards.

This study has demonstrated that V. arboreum traits can be incorporated into cultivated highbush and provide viable, fertile progeny with traits that are useful in blueberry breeding.

APPENDIX

Table 36. Leaf length for the taxa used in this study.

Taxa	Number of plants	Reps per plant	Mean (mm)	Range (mm)	Variance[z] (mm^2)
V. darrowi	20	5	10.9	8.1-14.3	3.2
V. arboreum	20	5	36.3	28.8-42.2	12.9
F_1	16	5	22.6	16.9-34.2	20.9
Highbush	20	5	45.1	37.6-56.2	19.1
MIK	20	5	38.6	31.5-46.1	17.6

[z]Variance of the means.

Table 37. Leaf width for the taxa used in this study.

Taxa	Number of plants	Reps per plant	Mean (mm)	Range (mm)	Variance[z] (mm^2)
V. darrowi	20	5	5.2	3.1-7.1	1.4
V. arboreum	20	5	22.5	16.6-27.7	9.0
F_1	16	5	9.6	6.8-16.6	5.4
Highbush	20	5	24.4	17.8-31.4	8.9
MIK	20	5	18.4	15.4-21.3	3.4

[z]Variance of the means.

Table 38. Leaf length from the base to the widest point for the taxa used in this study.

Taxa	Number of plants	Reps per plant	Mean (mm)	Range (mm)	Variance[z] (mm^2)
V. darrowi	20	5	6.4	4.7-9.4	1.6
V. arboreum	20	5	20.8	16.1-25.2	6.6
F_1	16	5	13.3	9.2-19.8	8.2
Highbush	20	5	22.1	18.3-29.6	5.9
MIK	20	5	21.6	17.6-26.6	5.1

[z]Variance of the means.

Table 39. Leaf shape for the taxa used in this study. Leaf shape was calculated by dividing the length of the leaf by the distance from the base to the widest point.

Taxa	Number of plants	Reps per plant	Mean (mm)	Range (mm)	Variance[z] (mm^2)
V. darrowi	20	5	1.71	1.52-1.96	0.01
V. arboreum	20	5	1.80	1.60-1.90	0.00
F_1	16	5	1.72	1.57-2.06	0.01
Highbush	20	5	2.05	1.81-2.35	0.02
MIK	20	5	1.79	1.65-1.94	0.00

[z]Variance of the means.

Table 40. Corolla length for the taxa used in the study.

Taxa	Number of plants	Reps per plant	Mean (mm)	Range (mm)	Variance[z] (mm^2)
V. darrowi	20	5	6.0	4.8-7.4	0.3
V. arboreum	20	5	6.7	5.5-7.6	0.4
F_1	15	5	7.6	6.7-8.8	0.4
Highbush	20	5	10.3	9.3-11.5	0.3
MIK	20	5	9.9	8.4-11.7	0.5

[z]Variance of the means.

Table 41. Corolla width for the taxa used in the study.

Taxa	Number of plants	Reps per plant	Mean (mm)	Range (mm)	Variance[z] (mm^2)
V. darrowi	20	5	3.4	3.0-4.1	0.1
V. arboreum	20	5	5.6	4.6-6.7	0.3
F_1	15	5	3.8	3.1-4.5	0.2
Highbush	20	5	6.2	5.4-7.0	0.3
MIK	20	5	5.4	4.7-7.3	0.3

[z]Variance of the means.

Table 42. Corolla aperture for the taxa used in the study.

Taxa	Number of plants	Reps per plant	Mean (mm)	Range (mm)	Variance[z] (mm^2)
V. darrowi	20	5	1.7	1.3-2.2	0.1
V. arboreum	20	5	5.0	4.1-6.0	0.3
F_1	15	5	2.3	1.8-3.2	0.2
Highbush	20	5	3.2	2.6-4.0	0.2
MIK	20	5	3.1	2.5-4.2	0.2

[z]Variance of the means.

Table 43. Pedicel length for the taxa used in this study.

Taxa	Number of plants	Reps per plant	Mean (mm)	Range (mm)	Variance[z] (mm^2)
V. darrowi	20	5	4.3	2.5-5.5	0.6
V. arboreum	20	5	11.6	7.8-15.8	3.9
F_1	15	5	6.7	3.1-9.1	3.4
Highbush	20	5	4.2	2.6-7.1	0.8
MIK	20	5	5.8	3.4-8.1	1.7

[z]Variance of the means.

Table 44. Peduncle length for the taxa used in this study.

Taxa	Number of plants	Reps per plant	Mean (mm)	Range (mm)	Variance[z] (mm^2)
V. darrowi	20	5	4.1	2.2-8.0	1.8
V. arboreum	20	5	44.3	28.6-70.4	123.3
F_1	15	5	15.8	3.9-26.2	42.7
Highbush	20	5	9.1	4.4-13.2	6.5
MIK	20	5	11.3	7.2-18.0	11.4

[z]Variance of the means.

Table 45. Bracteole length for the taxa used in the study.

Taxa	Number of plants	Reps per plant	Mean (mm)	Range (mm)	Variance[z] (mm^2)
V. darrowi	14	5	1.7	1.4-2.2	0.06
V. arboreum	4	5	1.7	1.5-2.0	0.04
F_1	9	5	2.7	2.1-3.9	0.41
Highbush	20	5	3.8	3.2-4.9	0.17
MIK	20	5	3.5	2.5-4.1	0.17

[z]Variance of the means.

Table 46. Bracteole width for the taxa used in the study.

Taxa	Number of plants	Reps per plant	Mean (mm)	Range (mm)	Variance[z] (mm^2)
V. darrowi	14	5	1.1	0.95-1.35	0.0200
V. arboreum	4	5	0.3	0.30-0.25	0.0006
F_1	9	5	0.9	0.50-1.35	0.0619
Highbush	20	5	2.2	1.40-2.75	0.0121
MIK	20	5	1.5	1.05-1.95	0.0759

[z]Variance of the means.

Table 47. Berry weight for the taxa used in this study. Each replication was composed of 15 berries. Values are expressed as single berry weights.

Taxa	Number of plants	Reps per plant	Mean (g)	Range (g)	Variance[z] (g^2)
V. darrowi	20	2	0.20	0.12-0.30	0.0018
V. arboreum	20	2	0.28	0.18-0.45	0.0044
F_1	7	2[y]	0.20	0.16-0.26	0.0011
Highbush	20	2	1.65	1.19-2.10	0.0586
MIK	20	2	0.77	0.54-1.45	0.0482

[z]Variance of the means.
[y]2 plants had only 1 replication.

Table 48. Large seed weight for the taxa used in this study. Each replication is composed of 15 seeds.

Taxa	Number of plants	Reps per plant	Mean (mg)	Range (mg)	Variance[z] (mg^2)
V. darrowi	20	2	7.28	5.6-9.3	1.00
V. arboreum	20	2	20.35	14.3-25.3	9.35
F_1	3	2	19.61	12.6-28.9	46.79
Highbush	20	2	10.40	8.8-13.0	1.56
MIK	20	2	14.80	9.5-18.3	3.78

[z]Variance of the means.

Table 49. Pollen stainability for the taxa used in this study. A minimum of 200 grains per plant were examined using aceto-carmine stain. Pollen from 2 flowers was mixed together.

Taxa	Number of plants	Reps per plant	Mean (%)	Range (%)	Variance
V. darrowi	20	1	84.9	45.0-99.0	232.6
V. arboreum	20	1	78.3	21.4-100.0	478.2
F_1	15	1	0.9	0.0-5.0	2.1
Highbush	20	1	87.6	64.9-100.0	102.8
MIK	64	1	43.7	0.0-92.1	930.6
(MIK x HB)	111	1	75.1	0.0-99.0	389.8
(MIK x MIK)	56	1	45.8	0.0-97.5	987.2
(MIK selfed)	16	1	48.8	0.0-75.0	464.3

Table 50. Amount of pollen per flower for the taxa used in this study. A scale of 0-5 was used with 0 being no pollen and 5 being the highest amount.

Taxa	Number of plants	Reps per plant	Mean	Range	Variance
V. darrowi	20	1	4.2	3-5	0.46
V. arboreum	20	1	2.7	1-5	1.33
F_1	15	1	1.7	1-3	0.46
Highbush	20	1	4.2	2-5	0.85
MIK	64	1	2.5	0-5	1.78
(MIK x HB)	111	1	3.2	0-5	1.02
(MIK x MIK)	56	1	2.5	0-5	0.92
(MIK selfed)	16	1	2.4	1-4	0.62

LITERATURE CITED

Aalders, L.E. and I.V. Hall. 1961. Pollen incompatibility and fruit set in lowbush blueberries. Can. J. Genet. Cytol. 3:300-307.

Ahokas, H. 1979. Artificial reciprocal hybrids between Vaccinium microcarpum and V. vitis-idaea. Ann. Bot. Fenn. 16:3-6.

Allgood, W.L. 1970. A bizarre huckleberry tree. Amer. Hort. Magazine 49:84-85.

Ballinger, W.E., E.P. Maness and J.R. Ballington. 1982. Anthocyanins in ripe fruit of the sparkleberry, Vaccinium arboreum Marsh. Can. J. Plant Sci. 62:683-687.

Ballington, J.R. 1980. Crossability between subgenus Cyanococcus (Gray) Klotzsch and subgenus Polycodium (Raf.) Sleumer in Vaccinium. HortSci. 15:419.

Ballington, J.R. 1990. Germplasm resources available to meet future needs for blueberry cultivar improvement. Fruit Var. J. 44:54-62.

Ballington, J.R. and G.J. Galletta. 1978. Comparative crossability of 4 diploid Vaccinium species. J. Amer. Soc. Hort. Sci. 103:554-560.

Briggs, F.N. and P.F. Knowles. 1967. Introduction to plant breeding. Reinhold Publishing Corp. New York.

Camp, W.H. 1942. On the structure of populations in the genus Vaccinium. Brittonia. 4(2):189-204.

Camp, W.H. 1945. The North American blueberries with notes on other groups of vacciniaceae. Brittonia. 5(3):203-275.

Cockerham, L.E. and G.J. Galletta. 1976. A survey of pollen characteristics in certain Vaccinium species. J. Amer. Soc. Hort. Sci. 101:671-676.

Coville, F.V. 1927. Blueberry chromosomes. Sci. 66:565-566.

Coville, F.V. 1937. Improving the wild blueberry. In: Yearbook of agriculture. USDA. Washington.

Darrow, G.M. 1941. Seed size in blueberry and related species. Proc. Amer. Soc. Hort. Sci. 38:438-450.

Darrow, G.M. 1960. Blueberry breeding: past present future. Amer. Hort. Mag. 39(1):15-33.

Darrow, G.M. and W.H. Camp. 1945. Vaccinium hybrids and the development of new horticultural material. Bul. Torrey Bot. Club. 72:1-21.

Dweikat, I.M. and P.M. Lyrene. 1988. Adventitious shoot production from leaves of blueberry cultured in vitro. HortSci. 23:629.

Dweikat, I.M. and P.M. Lyrene. 1989a. Response of highbush blueberry seed germination to gibberellin A_3 and ^{6}N-benzyladenine. Can. J. Bot. 67:3391-3393.

Dweikat, I.M. and P.M. Lyrene. 1989b. Production and evaluation of a synthetic hexaploid in blueberry. Theo. Appl. Genet. 77:799-804.

Eck, P. and C.M. Mainland. 1971. Highbush blueberry fruit set in relation to flower morphology. HortSci. 6:494-495.

El-Agamy, S.Z.A., W.B. Sherman and P.M. Lyrene. 1981. Fruit set and seed number from self- and cross-pollinated highbush (4x) and rabbiteye (6x) blueberries. J. Amer. Soc. Hort. Sci. 106:443-445.

Galletta, G.J. 1975. Blueberries and cranberries. In: Advances in fruit breeding. J. Janick and J. N. Moore (Eds). Perdue Univ. Press. West Lafayette, Indiana.

Galletta, G.J. and A.S. Fish, Jr. 1971. Interspecific blueberry grafting, a way to extend Vaccinium culture to different soils. J. Amer. Soc. Hort. Sci. 96:294-298.

Garvey, E.J. and P.M. Lyrene. 1987. Self incompatibility in 19 native blueberry selections. J. Amer. Soc. Hort. Sci. 112:856-858.

Godfey, R. K. 1988. Trees, shrubs, and woody vines of northern Florida and adjacent Georgia and Alabama. University of Georgia Press. Athens and London.

Hadley, H.H. and S.J. Openshaw. 1980. Interspecific and intergeneric hybridization. In: Hybridization of crop plants. W.R. Fehr and H.H. Hadley (Eds). Amer. Soc. of Agro. and Crop Sci. Soc. of Amer. Madison, Wisconsin.

Harrison, R.E., J.J. Luby and P.D. Ascher. 1993. Genetic characteristics of self fertility in highbush and half-high blueberries. Euphytica. 67:79-88.

Hartmann, H.T. and D.E. Kester. 1975. Plant propagation. Third edit. Prentice-Hall, Inc. Englewood Cliffs, New Jersey.

Haywood, J.D. 1994. Seed viability of selected tree, shrub, and vine species stored in the field. New For. 8:143-154.

Hermsen, J.G.Th. 1992. Introductory considerations on distant hybridization. In: Distant hybridization of crop plants. G. Kalloo and J. B. Chowdhury (Eds). Springer-Verlag. Berlin.

Hiirsalmi, H. 1977. Inheritance of characters in hybrids of Vaccinium uliginosum and highbush blueberries. Ann Agric Fenn. 16:7-18.

Hiirsalmi, H. and A. Lehmushovi. 1982. A Finnish highbush blueberry variety 'Aron'. Ann. Agric. Fenn. 21(4):151-154.

Hochberg, Y. 1987. Multiple comparison procedures. John Wiley and Sons. New York.

Krebs, S.L. and J.E. Hancock. 1988. The consequences of inbreeding on fertility in Vaccinium corymbosum L. J. Amer. Soc. Hort. Sci. 113:914-918.

Krebs, S.L. and J.E. Hancock. 1990. Early-acting inbreeding depression and reproductive success in the highbush blueberry, Vaccinium corymbosum L. Theo. Appl. Genet. 79:825-832.

Laverty, T. and N. Vorsa. 1991. Fertility of aneuploids between the 5x and 6x levels in blueberry: the potential for gene transfer from 4x to 6x levels. J. Amer. Soc. Hort. Sci. 116:330-335.

Luby, J.J., J.R. Ballington, A.D. Draper, K. Pliszka, and M. E. Austin. 1990. Blueberries and cranberries (Vaccinium). In: Genetic resources of temperate fruit and nut crops 1. J.N. Moore and J. R. Ballington, Jr. (Eds). International Society for Horticulture Science. Wageningen, The Netherlands.

Lyrene, P.M. 1986. Variation within Vaccinium darrowi blueberry in Florida. HortSci. 21:512-514.

Lyrene, P.M. 1988. Fecundity of crosses between tetraploid and hexaploid Vaccinium. J. Amer. Hort. Sci. 113:592-595.

Lyrene, P.M. 1989. Pollen source influences fruiting of 'Sharpblue' blueberry. J. Amer. Soc. Hort. Sci. 114:995-999.

Lyrene, P.M. 1989. The Florida climate as it relates to blueberry production. Proc. Fla. State Hort. Soc. 102:208-212.

Lyrene, P.M. 1991. Fertile derivatives from sparkleberry x blueberry crosses. J. Amer. Soc. Hort. Sci. 116:899-902.

Lyrene, P.M. 1993. Some problems and opportunities in blueberry breeding. Acta. Hort. 346:63-71.

Lyrene, P. M. and J. R. Ballington, Jr. 1986. Wide hybridization in Vaccinium. HortSci. 21:52-57.

Lyrene, P.M. and W.B. Sherman. 1978. Blueberry cultivars for Florida. Fruit Var. J. 32:9-12.

Lyrene, P.M. and W.B. Sherman. 1983. Mitotic instability and 2n gamete production in Vaccinium corymbosum x V. elliottii hybrids. J. Amer. Soc. Hort. Sci. 108:339-342.

Lyrene, P.M. and W.B. Sherman. 1992. The 'Sharpblue' southern highbush blueberry. Fruit Var. J. 46:194-192.

Meader, E.M. and G.M. Darrow. 1944. Pollination of the rabbiteye blueberry and related species. Proc. Amer. Soc. Hort. Sci. 45:267-274.

Megalos, B.S. and J.R. Ballington. 1987. Pollen viability in five southeastern United States diploid species of Vaccinium. J. Amer. Soc. Hort. Sci. 112:1009-1012.

Moore, G.A. and G.B. Collins. 1983. New challenges confronting plant breeders. In: Isozymes in plant genetics and breeding, part A. S.D. Tanksley and T.J. Orton (Eds.). Elsevier. Amsterdam.

Moore, J.N. 1965. Improving highbush blueberries by breeding and selection. Euphytica. 14:39-48.

Moore, J.N. 1966. Breeding. In: Blueberry culture. P. Eck (Ed). Rutgers Univ. Press. New Brunswick, New Jersey.

Nielsen, G. 1985. The use of isozymes as probes to identify and label plant varieties and cultivars. Isozymes: current topics in biological and medical research. 12:1-32.

Ortiz, R., L.P. Bruederle, and T. Laverty. 1992. Occurrence of unreduced pollen in diploid blueberry species, Vaccinium sect. Cyanococcus. Theor. Appl. Genet. 85:55-60.

Pliszka, K., E. Czesnik and H. Karwowska. 1980. Interspecific highbush blueberry hybridization and selection for winter hardiness and other characters. In: Proc. 4th N. Amer. blueberry res workers conf., Fayetteville, AR.

Radford, A.E., W.C. Dickison, J.R. Massey and C.R. Bell. 1974. Vascular plant systematics. Harper & Row. New York.

Rieseberg, L.G. 1995. The role of hybridization in evolution: old wine in new skins. Amer. J. Bot. 82:944-953.

Rousi, A. 1963. Hybridization between Vaccinium uliginosum and cultivated blueberry. Ann. Agric. Fenn. 23:12-18.

Rousi, A. 1966. Cytological observations on some species and hybrids of Vaccinium. Zuchter 36:352-359.

Soltis, D.E., C.H. Haufler, D.C. Darrow, and G.J. Gastony. 1983. Starch gel electrophoresis of ferns: a compilation of grinding buffers, gel and electrode buffers, and staining schedules. Amer. Fern J. 73:9-27.

Stebbins, G.L. Jr. 1950. Variation and evolution in plants. Columbia Univ Press. New York.

Stebbins, G.L. 1958. The inviability, weakness and sterility of interspecific hybrids. In: Advances in genetics. E. M. Demeric (Ed). Academic Press Inc. New York.

Stockton, L.A. Jr. 1976. Propagation and autecology of Vaccinium arboreum and its graft compatibility with Vaccinium ashei. M.S. Thesis. Texas A & M Univ. College Station.

van Heemstra, M.I., L.P. Bruederle, and N. Vorsa. 1991. Inheritance and linkage of thirteen polymorphic isozyme loci in diploid blueberry. J. Amer. Soc. Hort. Sci. 116:89-94.

Vander Kloet, S.P. 1978. The taxonomic status of Vaccinium pallidum, the hillside blueberries including Vaccinium vacillans. Can. J. Bot. 56:1559-1574.

Vander Kloet, S.P. 1980. The taxonomy of the highbush blueberry, Vaccinium corymbosum. Can. J. Bot. 58:1187-1201.

Vander Kloet, S.P. 1983. The taxonomy of Vaccinium section Cyanococcus: a summation. Can. J. Bot. 61:256-266.

Vander Kloet, S.P. 1988. The genus Vaccinium in North America. Pub. 1828. Can. Govt. Pub. Ctr. Ottawa.

Vander Kloet, S.P. 1991. The consequences of mixed pollination on seed set in Vaccinium corymbosum. Can. J. Bot. 69:2448-2454.

Vander Kloet, S.P. and P.M. Lyrene. 1987. Self-incompatibility in diploid, tetraploid, and hexaploid Vaccinium corymbosum. Can. J. Bot. 65:660-665.

Vorsa, N., G. Jelenkovic, A.D. Draper, and W.V. Welker. 1987. Fertility of 4x x 5x and 5x x 4x progenies derived from Vaccinium ashei/corymbosum pentaploid hybrids. J. Amer. Soc. Hort. Sci. 112:993-997.

Vorsa, N., P.S. Manos, and M.I. van Heemstra. 1988. Isozyme variation and inheritance in blueberry. Genome. 30:776-781.

Weeden, N.F. 1989. Applications of isozymes in plant breeding. In: Plant breeding reviews vol 6. J. Janick (Ed). Timber Press. Portland, Oregon.

Wendel, J.F. and N.F. Weeden. 1989. Visualization and interpretation of plant isozymes. In: Isozymes in plant biology. D.E. Soltis and P.S. Soltis (Eds). Dioscorides Press. Portland, Oregon.

White, E. and J.H. Clark. 1939. Some results of self-pollination of the highbush blueberry at Whitesbog, New Jersey. Proc. Amer. Soc. Hort. Sci. 36:305-309.

Wilson, P. 1992. On inferring hybridity from morphological intermediacy. Taxon. 41:11-23.

Wood, G.W. 1968. Self-fertility in the lowbush blueberry. Can. J. Plant Sci. 48:431-433.

BIOGRAPHICAL SKETCH

Sylvia J. Brooks was born in Baltimore, Maryland, but was raised in Fayetteville, Arkansas. With her family, she spent a year in England in 1968 and a year in Japan in 1972. She graduated from Fayetteville High School in 1976. After high school, she attended the University of Utah for a year and then the University of Arkansas for a year. In 1979, she married and moved to Houston, Texas. Her daughter was born there in 1981. In 1984, she returned to Fayetteville, Arkansas. She received a Bachelor of Science degree in botany from the University of Arkansas in 1988. In 1991, she obtained a Master of Science degree in agricultural science, also from the University of Arkansas. Her master's research dealt with the determination and characterization of sugars in several peach breeding lines. She currently resides in Gainesville, Florida, where she is pursuing her Ph.D. in horticultural sciences, specializing in plant breeding.

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a thesis for the degree of Doctor of Philosophy.

Paul Lyrene
Paul Lyrene, Chair
Professor of Horticultural Science

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a thesis for the degree of Doctor of Philosophy.

Walter S. Judd
Walter S. Judd
Professor of Botany

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a thesis for the degree of Doctor of Philosophy.

Gloria A. Moore
Gloria A. Moore
Professor of Horticultural Science

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a thesis for the degree of Doctor of Philosophy.

Kenneth H. Quesenberry
Kenneth H. Quesenberry
Professor of Agronomy

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a thesis for the degree of Doctor of Philosophy.

Wayne B. Sherman
Wayne B. Sherman
Professor of Horticultural Science

This thesis was submitted to the Graduate Faculty of the College of Agriculture and to the Graduate School and was accepted as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

May, 1996

Jack L. Fry

Dean, College of Agriculture

Dean, Graduate School